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# Thomas Addis Emmet

## Presidential address

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FOR the traditional presidential address I have chosen to pay tribute to Thomas Addis Emmet, who made significant contributions to the founding of modern gynecology and who, by his precepts, reduced some of the hazards of obstetrics as practiced in his day. Thomas Addis Emmet was born 133 years ago. Although his forebears had lived in Ireland and had been ardent Irish patriots for over 100 years, Emmet stated that his background was entirely English. His ancestors on both sides of his family had emigrated from England to Ireland and to the United States. The Emmet family can be traced back through four consecutive generations of noteworthy physicians and surgeons to Christopher Emmet who was born in 1700 and practiced medicine in Tipperary, Ireland.

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Thomas Addis Emmet was a great-nephew of the famous Irish patriot, Robert Emmet, and a grandson of the exiled Thomas Addis Emmet, who won distinction in medicine, law, and politics, and in business as a railroad promoter. When the grandfather emigrated to New York City in 1804 he brought his wife and 3 of his children including John Patten Emmet, a 10-year-old boy who was to become the father of the American gynecologist.

John Patten Emmet, the father of Thomas Addis, had many scientific and cultural attainments including proficiency in mathematics, an extensive knowledge of Latin and Greek, fluency in French and Italian, and considerable knowledge of German. He was also well-versed in English literature, and, for his day, he was an accomplished scientist. After he had recovered from tuberculosis, which had terminated his training as a cadet at West Point before he could be graduated, he won his degree as a physician at the

Columbia University College of Physicians and Surgeons. After 3 years at the practice of medicine at Charleston, South Carolina, he was appointed by Thomas Jefferson to the faculty at the University of Virginia, first as Professor of Natural History and later as Professor of Chemistry and Materia Medica. Soon after he joined the faculty at the University of Virginia, he married Miss Mary Byrd Farley Tucker, the niece of a professor at the University. She was a native of Bermuda and a member of the well-known Byrd and Tucker families of Virginia.

Thomas Addis Emmet was born at Charlottesville, Virginia, on May 29, 1828. His boyhood was spent on his father's 100 acre farm near the University. As a healthy, robust boy he learned, in addition to his duties at the farm, to use firearms and, accompanied by his faithful dog, he spent much time hunting birds, rabbits, and rodents. He was not a studious boy and when the local school failed to inspire his interest in learning, arrangements were made for him to attend elementary classes at a school in Charlottesville and also to be tutored privately for several years. At 11 years of age he was enrolled as one of four or five hundred boys at St. Thomas Hall, a school near Flushing, Long Island. His unhappy experience at this school ended during the second year when for economic reasons the school closed. During the same year his father had a recurrence of tuberculosis and died.

The next 5 years were extremely frustrating for the young Thomas Addis. After the father's estate had been settled the family lacked the necessary funds to permit him to continue his formal education. For some time he spent several hours every day studying various subjects in the library of a great-uncle, Saint George Tucker, a Professor of Economics and Belles Lettres at the University of Virginia, who also examined him on his reading.

At 16 years of age he managed to enroll as a student at the University of Virginia. For various reasons this effort to continue his formal education failed. Eventually his mother was shocked to receive a notice informing her that her son's name had been dropped from the student roll at the University.

At 18 years of age, he enrolled as a student at the Jefferson Medical College. This was made possible with the assistance of Dr. Robley Dunglison who was a friend of the family and a former colleague of John Emmet at the University of Virginia. Thomas was aware of the scholastic deficiencies in his background and was somewhat concerned as to whether he could keep up with studies prescribed by the faculty at the medical college. He had always been able to learn more readily from observation and listening than from formal studies at school. From his exceptional parents and home life he had acquired intellectual discipline, a fairly broad education, and the gracious manners and attitudes expected of the well-bred young man of his day.

Emmet's studies at the Medical College began with a lecture by Dr. J. K. Mitchell, Professor of the Theory and Practice of Medicine. He was the father of S. Wier Mitchell, one of Emmet's classmates and lifelong friends. Emmet was fascinated by the first lecture he had heard and was at once convinced that his life's work was laid out for him. He was soon able to study to good advantage and throughout the 4 year medical course his interest in subjects taught never waned. He was surprised and pleased to find that his ability to understand and to remember what he had seen and heard surpassed that of some of his classmates who had had the benefit of a formal premedical college education. His studies were interrupted twice by illness, first by an attack of smallpox and later by pneumonia. He attributed his rapid recovery from pneumonia to prompt bleeding and calomel.

Time spent at studying medicine passed happily and brought genuine satisfaction from his ability to maintain a good scholastic record and from the fact that he had found a cause to which he could dedicate his life. He stated that he was graduated from medi-

cal college without having dissected a muscle or nerve in the body and without having written a single prescription or having attended an obstetric case. He was well aware of the importance of having practical experience in treating patients. Soon after he was graduated he accepted an appointment on the Staff of the Emigrant Refuge Hospital on Ward's Island in New York City. This institution, which eventually had a 4,000 bed capacity, was filled for the most part by Irish emigrants who left their native land during the 1847-1848 famine. By the hundreds they were transferred from ships to the Emigrant Refuge Hospital, suffering from severe malnutrition, typhus fever, smallpox, and cholera. It was at this hospital that Emmet had his first practical experience in the treatment of patients. In the course of 3½ years, first as Resident Physician and later as Visiting Physician, he was made responsible for the care of a total of 11,000 patients with a great variety of conditions including all of the "eruptive" fevers of adults and children and over 1,900 cases of typhus fever in adult men. Other activities at the hospital included a considerable number of operations and at least 5 obstetric deliveries per day. Emmet personally performed 1,000 postmortem examinations. He was always overworked and during his term of service had to be relieved of his duties for a total of 3½ months to recover from two attacks of typhus fever and a severe attack of rheumatic fever. His service at this hospital ended abruptly when a change in politics resulted in complete replacement of the professional staff.

On Feb. 14, 1854, at 25 years of age Emmet married Miss Catherine R. Duncan, a daughter in a prominent family with a Scottish background, in a community near Montgomery, Alabama.

During a cold wintry evening early in the spring of 1855, while tabulating the records of typhus fever patients treated at Ward's Island, he had a surprise visit from Dr. Marion Sims. On the way home from a meeting of the Board of Governors of the new Woman's Hospital, the horse-drawn trolley car in which Sims was riding had become derailed. Emmet did not at once recognize the visitor who had rapped on his window and had requested permission to come inside to get warm. Sims introduced himself and immediately became interested in the project at which Emmet was working late at night. After he explained what he was doing Sims told him he was exactly the one he needed to help with his work at the new Woman's Hospital. Emmet accepted Sims' invitation to observe, the following morning, one of his operations for a serious birth injury. He had never before seen such an operation and was obviously fascinated by the condition and operative procedure which he had come to observe.

The Woman's Hospital was founded primarily to provide an opportunity for J. Marion Sims to perform the operation which he had perfected for vesicovaginal fistula. Its service to the public began in a rented private home at 83 Madison Avenue, New York, which had been converted to accommodate 40 patients. Soon after its doors were opened on May 4, 1855, all of its beds were filled and Sims was operating upon nearly one vesicovaginal fistula per day. It became apparent that he needed an assistant. He was obviously not satisfied by the Board of Governors' decision to fill this need by the appointment of a woman physician. Emmet was, therefore, persuaded to serve as his assistant for about 6 months without an official appointment, that is, until the fall of 1855, when he was made an Assistant Surgeon.

During the next 5½ years Emmet assisted Sims at all of his operations. He mastered his technique for closure of fistulas and applied what he had learned by performing personally an increasing number of operations. In fact it was not long before he was doing two thirds of all the operations performed at the Hospital. In addition to his work as a surgeon he assumed an active role in administering all of the hospital's routine affairs. He kept records in longhand of all patients admitted to the hospital, including their clinical histories, detailed descriptions

of conditions for which they had operations, and accurate accounts of all operations performed. These clinical records in bound volumes are on display at the Woman's Hospital. They contain many of Emmet's quite artistic, colored pen and ink drawings of extensive fistulas and other gynecologic conditions which were treated.

There can be no doubt that Sims was an ingenious, skillful surgeon. In the development of a new field of surgery he was never at a loss to devise techniques or modifications in techniques to suit variations in conditions which he encountered. In fact Emmet stated that it was unusual for Sims to use the same surgical procedure to treat almost identical conditions. Emmet's approach was a more analytical and methodical one. He was inclined to adhere to surgical procedures which had proved themselves to be sound and efficient, but he did devise some improvements in the procedures which he had learned from Sims.

Within a few months after the Woman's Hospital began its service to the public it became apparent that its original makeshift quarters were entirely inadequate to meet the increasing demand of patients for treatment. Sims found it necessary to devote much time and energy at conferences and at lobbying, wire-pulling, and other activities connected with the hospital's urgent need for expansion. It was for this reason that Emmet had to assume a major portion of responsibility for conduct of the hospital's affairs. Through Sim's determined efforts the hospital acquired a state charter, financial support from the state, a grant of land from the city on which to construct a new building, and much wider support from many influential people in the community.

With approach of the Civil War the hospital passed through a crisis in 1861 which might have terminated its existence. When war was declared, Emmet set out for Montgomery, Alabama, to offer his services to Jefferson Davis as an army surgeon. His offer was politely declined and without delay he complied with the recommendation that he return to New York to look after his family and to carry on with his professional work. During Emmet's absence Sims persuaded a brilliant young Southerner, T. Gaillard Thomas, to run the Outpatient Clinic.

Conditions resulting from the war created a difficult situation for Sims, which was aggravated by his own tactless expressions of sympathy for the Southern cause. His active private practice, composed largely of patients from the South, dwindled ominously when many returned to their native states below the Mason-Dixon line. Efforts to get financial support for the Hospital had become increasingly difficult and as a result of the war Sims had lost favor with the medical profession. He was in a quandary as to what course to follow. With conviction that the hospital's affairs could be safely left to Emmet and Thomas and with the excuse that he needed a vacation he prepared to leave for Europe in June, 1861.

Sims' decision to leave at such a critical time was a profound shock to members of the Board of Governors. They doubted that the hospital could survive without his determined personal efforts to meet its needs. Serious consideration was given to suspending its activities.

Reluctance on the part of the Board of Governors to appoint Emmet to fill Sims' position was probably not due to lack of confidence in his capabilities. Throughout his connection with the hospital he had concentrated all of his time and energy on its professional work and administrative problems. He had apparently never had any special interest in efforts to ingratiate himself with the Board of Governors or the Board of Lady Managers. By contrast, Sims had held a "magnetic sway" over these official bodies. They had come to think of him not only as the founder of the hospital but in fact as the Woman's Hospital itself. Some rather vehement opposition to Emmet's appointment had arisen also from the fact that he had left the Episcopalian church and had become an ardent Roman Catholic convert. His wife was a Roman Catholic and he wished his children to have a com-

mon religious family background. All objections to his appointment were finally overcome and, at 33 years of age, he was made Chief Surgeon to the hospital.

Emmet's new appointment brought him genuine satisfaction. He realized that it offered a unique opportunity to promote the development of gynecology and to teach what had been learned about the new field of surgery. His association with Sims, described as like that of a father and son, had always been a most congenial one. However, he had apparently been disturbed by the fact that his own professional standing and accomplishments had been overshadowed by the charm of Sims' personality and the brilliance of his surgical achievements.

When Emmet assumed his duties as Chief Surgeon he was well on the way to becoming a master at vaginal plastic surgery. He had acquired confidence in his own ability as a surgeon and he welcomed the opportunity to prove himself. He refused to be daunted by many difficult problems, including the Board of Governor's obvious lack of enthusiasm in appointing him to fill the position vacated by Sims. At considerable sacrifice he gave up obstetrics and a lucrative general practice in order to devote all of his time and energy to his hospital duties. He established two clinics per week at which members of the medical profession had opportunities to learn about the successful surgical techniques which had been devised. These clinics were held for many years and were attended eventually by more than 1,000 physicians per year from all parts of the United States and from abroad. New techniques were taught by Emmet's careful descriptions, his very good drawings on a blackboard, and by observation of the various operations which he performed.

At the time that Sims and Emmet started their work at the Woman's Hospital, in 1855, gynecology as a specialized field of surgery did not exist. For 12 years they carried on their pioneer work in gynecology at the hospital's original makeshift quarters. Operations were performed with patients in a knee-chest or semiprone position on a plain wooden table. The Sims speculum, held by an assistant, was used to expose the field of operation. Until near the end of this time they had no knowledge of the bacterial origin of infections. For success of wound healing they depended upon scrupulous cleanliness. The operative field, surgical instruments, and the surgeon's arms and hands were scrubbed vigorously with hot water and turpentine soap. Clean linen was used to drape the patient. Anesthesia was not used at the hospital during the first 10 years, that is, until about 1865. Operating time had to be reduced to a minimum to keep within the physical endurance of the patients. There were no reliable precedents for the surgical techniques which they devised. Instruments had to be designed and constructed. With the assistance of poorly trained domestic servants they had to assume full responsibility for the postoperative care of their patients. There were no trained nurses. In view of all of these handicaps their accomplishments were truly remarkable.

By the time Emmet assumed his duties as Chief Surgeon in 1861, the efficiency of Sims' technique for closure of vaginal fistulas had been established. More or less successful methods for the diagnosis and treatment of many abnormal pelvic conditions had been developed and the foundation of modern gynecology had been laid.

Emmet made many significant contributions to the development of obstetrics and gynecology. The success of the classical Sims operation for closure of fistulas was apparently increased by improvements in technique which he developed. He used wider areas of denudation along the margins of a fistula. When large fistulas could not be closed without undue tension he resorted to separation of the walls of the vagina and bladder, a procedure which in later years became known as "flap splitting." When the anterior vaginal wall was completely destroyed he occluded the vaginal introitus and established a suprapubic bladder fistula. Leakage of urine was prevented by pressure of a trusslike device over the

fistulous opening. This ingenious procedure led to some inconvenience for the patient who had to get on her knees and elbows to empty her bladder. In any event, 30 patients with fistulas which had been considered at first incurable or who had had previous unsuccessful operations were readmitted and cured by Emmet.

In 1868 Emmet published a report on the closure of 283 fistulas in patients treated before October, 1867, in the original 40 bed hospital. By 1900 this number had increased to 600.

He devised a plastic procedure for construction of a urethra in women in whom the urethra had been destroyed by birth injury. In later years he reported that he had performed 50 successful operations for this condition.

The butterfly denudation technique devised by Emmet for repair of relaxations and lacerations of the perineum and posterior vaginal wall was popular with gynecologists for many years.

Sims devised a plastic operation for uterine prolapse which involved only the anterior vaginal wall. Emmet found that the Sims technique could not be relied upon to cure prolapse unless it was combined with plastic procedures to correct any relaxations or lacerations of the perineum and posterior vaginal wall. He talked and wrote about uterine and pelvic support by connective tissue and fascia but probably did not have complete knowledge of structures which hold the uterus at a normal level in the pelvis.

Congenital absence of the vagina was successfully treated by use of a glass plug and gauze to maintain dilatation of a space opened between the bladder and rectum.

Emmet was always much interested in the importance of lacerations and chronic inflammatory lesions of the uterine cervix. He believed that they caused pelvic discomfort and that they predisposed to sterility, abortion, and cervical cancer. He was not the first to introduce surgical procedures to eliminate such conditions. After repairing cervical lacerations for many years by painstaking denudations and silver wire sutures he concluded that inflammatory lesions of the cervix could not be adequately relieved by trachelorrhaphy. He then resorted to amputation of the cervix. The technique which he adopted was his modification of an operation which Sims had used as early as 1859. After diseased tissue had been excised by superficial conization, flaps of vaginal mucous membrane were brought over the stump and fixed in position by sutures placed in a manner much like that which was popularized later by Sturmdorf. This prevented formation of hematomas beneath the flaps, a complication which had apparently forced Sims to abandon cervical amputation by his own technique.

As early as 1863, submucous uterine fibroids were removed vaginally by traction and when necessary by morcellation. After forward displacement of the bladder, fibroids on the anterior surface of the uterus were removed through an opening in the peritoneum of the anterior cul-de-sac.

His interest in inflammatory lesions of the pelvic organs led to efforts to differentiate between pelvic cellulitis, peritonitis, and adnexal infections. He recommended that pelvic infections be treated with medicated tampons and hot water douches. This was undoubtedly a welcome innovation for patients in the days when accepted treatment for such conditions had been intrauterine applications of various medicaments and ice water douches. He deserves credit also for adoption of wide colpotomy incisions to drain pelvic abscesses.

Both Sims and Emmet were much interested in so-called "uterine flexions" and their relation to pelvic discomfort, dysmenorrhea, abortion, and sterility. In 1876 Emmet presented a paper on this subject before this Society. It was actually the first paper to be presented at the first scientific meeting of the American Gynecological Society.

From the time Emmet began his service at the Woman's Hospital he became much interested in the cause and prevention of the serious birth injuries which came under treatment. From analysis of clinical records

he concluded that they were as a rule due to the pressure of prolonged labor and but rarely to the trauma of delivery by forceps. He condemned manipulation of the cervix to stimulate labor, stripping of the cervix over the presenting part, use of ergot to expedite the second stage of labor, and accouchement forcé. He advised delivery by forceps when the fetal head ceased to recede between uterine contractions and frequent catheterizations as needed during labor.

Obstetricians resented his intrusion into their field and decided that adoption of his recommendations would be "meddlesome obstetrics." They looked upon him as a gynecologic surgeon and emphasized his supposed lack of obstetric experience. Actually he had delivered some 1,100 babies, including our President Theodore Roosevelt. Eventually his advice was accepted and vaginal fistulas resulting from birth injuries were rarely encountered. At a later date when he became interested in cervical injuries he found it necessary to warn the obstetricians against use of forceps too early in the course of labor.

Under Emmet's direction the Hospital thrived and by 1866, 5 years after he became Chief Surgeon, the need for new quarters became urgent. Many of his friends made substantial contributions to a fund to build a modern hospital. Under Emmet's supervision, construction of a new building with accommodations for 75 patients was completed on Oct. 15, 1867. It was located at Fiftieth Street and Park Avenue on the same plot of ground which is now occupied by the Waldorf Astoria Hotel. In the new building, named the Wetmore Pavilion, a 40 bed ward was reserved for treatment of vaginal fistulas.

In 1872, after Emmet had served as Chief Surgeon for 11 years, the Board of Governors terminated his one-man rule. This change of policy was inspired by a marked increase in the number of patients admitted for treatment and to some extent by Emmet's arbitrary opinions regarding hospital management and staff appointments. By this time Sims had returned from Europe and was again well established in practice in New York City. The Board of Governors decided to appoint 4 gynecologists who would serve as a Medical Board and who would also assume equal shares of responsibility for the care and treatment of patients. The plan was put into effect by the appointment of J. Marion Sims, Thomas Addis Emmet, T. Gaillard Thomas, the Professor of Obstetrics and the Diseases of Women and Children at the College of Physicians and Surgeons, and Edmund Randolph Peaslee, the Professor of Gynecology at the Bellevue Hospital Medical College. In 1872 these were undoubtedly the 4 most eminent gynecologists in the United States.

After another 5 years the hospital's activities, under direction of its distinguished staff, had again outgrown its facilities and, in 1877, another new building, known as the Baldwin Pavilion, with accommodations for an additional 69 patients, was opened on the Fiftieth Street property. At about the same time three cottages were built near the new Pavilion to provide operating rooms, a recovery room for postoperative patients, and facilities for the care of patients who were seriously ill. It seems probable that one fundamental purpose of these cottages was to keep patients who had potential or active infections out of the main hospital buildings. In this new setup Listerism was also put into effect and as a result the incidence of wound infections, peritonitis, and septicemia was reduced.

Emmet was slow to accept the bacterial origin of infections. For operations by the vaginal route, he continued, as in the past, to depend upon absolute cleanliness for success of wound healing. Eventually he adopted the Lister routine for abdominal operations.

In 1883, the hospital's prestige was enhanced by the appointment of William Henry Welch as its first pathologist. After serving in this capacity for 3 years, he transferred to Bellevue and later to Johns Hopkins.

Three editions of Emmet's *Principles and*

*Practice of Gynecology* were published in this country and in England between 1879 and 1884. Translations of this text were also published in Germany and in France. For many years it was one of the accepted authorities on gynecology.

Emmet's approach to gynecologic problems was always a conservative one. He was consistently opposed to surgical operations until other forms of treatment had failed. All of his gynecologic operations were aimed at preserving or restoring functions of the pelvic organs. He believed vaginal plastic operations offered the best means of curing uterine prolapse. He was opposed to vaginal hysterectomy for prolapse unless the patient had uterine abnormalities, such as fibroids. He warned against indiscriminate removal of ovaries, and when possible he resected ovaries for ovarian cysts. He favored conservative treatment of pelvic inflammations and condemned removal of the tubes and ovaries for acute adnexal infections.

An impression that Emmet tried to avoid performing laparotomies is not entirely correct. There can be no doubt that his prime interest was in vaginal plastic surgery or that he preferred the vaginal approach for as many gynecologic operations as possible. He did his first abdominal operation in 1861, and at a meeting of this Society in 1900 he stated that he had done about 1,500 laparotomies. This is an impressive series when we consider that many of these operations were performed in days when techniques for abdominal operations were in an experimental stage and when mortality following such operations was at least 50 per cent.

In 1900, at 72 years of age, Emmet terminated his career in gynecology. After 45 consecutive years of service he resigned from the staff of the Woman's Hospital; he closed his 40 bed private hospital, and he retired from the practice of gynecology. During his professional career he made an enormous contribution to the care and treatment of charity patients; he carried on a large and lucrative private practice; he participated in the activities of various medical societies, and he found time to do an unusual amount of writing on scientific and other subjects.

He was a founder and a president of the New York Obstetrical Society. He was a founder and the sixth president of the American Gynecological Society. His contributions to programs of this Society included 10 original papers and discussions of some 40 scientific papers presented by Fellows of the Society. In addition to this about 60 of his monographs were published in American and foreign medical journals.

He held regular or honorary memberships in 25 scientific societies in this country and abroad. Other honors conferred upon him include a Doctor of Laws degree by the Jefferson Medical College in 1882, the Laetare Medal by the University of Notre Dame in 1899, and the insigne of the Knight Commander of the Order of St. Gregory the Great from Pope Pius X for his role as an eminent surgeon and a distinguished Catholic layman.

From the time Emmet was a medical student he maintained an active interest in Ireland's problems, which he attributed to oppression by the English. After he had retired from the practice of gynecology his 2 volume set of books entitled *Ireland under English Rule—or a Plea for the Plaintiff* was published in 1903. An enlarged second edition on this same subject was published in 1909. For 10 years he served as president of the Irish Federation of America.

He was also much interested in American history. For over 50 years he devoted great perseverance and ample means to his collection of Americana and eventually became one of the foremost and best-known collectors of manuscripts, prints, portraits, and autographs in this country. The value and extent of his collection can be judged by the fact that it was eventually sold for about $220,000. It contained 94 bound volumes of folio size, about 2,500 unbound letters and documents, 27 volumes of portraits, and 7 volumes of paper money. Most of his collection was donated to the Lenox Library and later was deposited at the New York Public Library.

In 1898 his extensive account of the Emmet family was published. At the time it was considered to be one of the best family histories ever written.

In 1911 he sold a valuable piece of property on Madison Avenue including his home for the erection of a 15 story office building. It was sold with the stipulation that a suite of rooms would be reserved for him on the top floor of the new building for the rest of his life. It was in this unusual abode surrounded by his books and historical documents that he spent the final 7 years of his life as a veritable recluse. By this time his devoted wife had died and his family was widely scattered in this country and abroad. He was also physically handicapped by deafness, poor healing of a fracture of one leg, and the infirmities of advancing age. Mentally, he continued to be active and alert. He devoted 8 hours a day to reading and writing until his death on March 1, 1919, at nearly 91 years of age.

Thus ended the life of a remarkable person. Through his contributions to the development and teaching of gynecology and to medical literature, he acquired, in his own right, the distinction of being one of the outstanding pioneers of modern gynecology.

# The histogenesis of acquired erosions of the cervix uteri

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ALTHOUGH the lesion variously known as erosion, eversion, or ectropion of the cervix uteri has been an important gynecologic problem for more than 100 years, no completely satisfactory explanation for its origin has been made. The purpose of this report is to review a number of the theories proposed in the past and their shortcomings and to direct attention to recent advances in our knowledge of the histology of the cervix uteri which may help to clarify the problem.

## Definition

The first difficulty in approaching this question is that of definition since a number of terms have been applied to different lesions with varying connotations. "Eversion" and "ectropion" are usually considered as referring to a turning or rolling out of the cervical canal, "much as the lining of a sleeve rolls out if it is too long for the cloth."[1] They are often confused with the "congenital erosion," and all three have been used as synonymous with "erosion." The term "erosion" itself finds different interpretations. At times, it is taken literally to indicate a loss of the surface epithelium, although most authors accept it as referring to an abnormality recognized on visual inspection and composed of histologic changes of the mucosa which correspond to the classic descriptions of Meyer.[6]

For the purposes of this essay, eversion, ectropion, and congenital erosion refer to lesions which basically are composed of columnar epithelium extending from the cervical canal over areas of the portio normally covered by squamous epithelium. The conditions under which this occurs must be discussed in more detail, and it will be seen that however this change is produced, it is essentially a first stage of an acquired "erosion" or "pseudoerosion."

## The clinical erosion

The term "erosion" has been used for over 100 years and was known to authors such as Lisfranc in 1841, Smith[3] in 1855, Churchill[2] in 1857, Joulin in 1861, and Bennet in 1864. Their descriptions indicate that they used the term "erosion" in its literal interpretation as meaning a loss of surface epithelium, although they differentiated it from deeper excoriations or ulcerations. They also recognized various types such as the aphthous, erythematous, herpetic, cockscomb granulation, vascular, pustular, papular, granular, and pemphigus. A familiar note is struck by Churchill's description of an erosion[2]: "When the inflamed cervix is brought into view by the speculum, its surface is found to offer a vivid red tinge instead of the pale rose color of health. It may present a uniform red hue and be dotted with florid papulae or with white pustules consisting of mucous

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glands, hypertrophied, or distended with muco-pus."

An erosion is readily recognized on visual inspection of the cervix. It assumes various sizes and shapes. It may be circular and surround the external os, it may be patchy, it may cover either the anterior or the posterior lip, and the edges may be sharp or irregular. It may extend from the external os for only a few millimeters or it may cover distances of several centimeters. Although the edges are sometimes shallower than the adjoining normal squamous epithelium, it must be clearly distinguished from ulcerations with a destruction and loss of tissue. The color is a characteristic intense red. The surface may be smooth but it also may have a granular appearance and may be traversed by low irregular folds of mucosa. Nabothian follicles may be visible within its limits or lie deep and protrude on the surface. When stained with the Schiller technique, some areas of the erosion do not take up the stain at all or only imperfectly so that it has a light mahogany color with patchy irregular light gray areas.

The readiness with which the eroded cervix may be exposed to view has made it a hunting ground for the proponents of examination with the colposcope, and indeed the examination of erosions under magnification serves to bring out details which are not clear to the naked eye.[4] Areas of ectopic columnar epithelium and "gland openings" in squamous epithelium are frequent findings. Of less frequent occurrence are Nabothian cysts, increased vascularization, isolated islands of columnar epithelium, and the three forms of colposcopic leukoplakia (true, ground, and mosaic patterns). In at least one study, a loss of surface tissue was demonstrable only occasionally when it was attributed to injury from instrumentation.[4]

Acquired erosions of the cervix occur during the reproductive period of life. They are found frequently during and after pregnancy, and in a previous study it was found that 55 per cent of a small series of obstetric patients had an erosion during the course of gestation and 82 per cent presented this lesion when seen 6 to 8 weeks post partum.[5] Although cervicitis is a frequent accompaniment, histologic examination often reveals no evidence of inflammatory changes in erosions.

### Histopathology

Any description of cervical erosion must differentiate between the so-called "congenital" and the "acquired" erosion. The congenital variety represents an area of the portio which is covered by columnar epithelium (Figs. 1 and 2), and this characteristic is also found in ectropion or eversion of the mucosa. It is significant that a congenital erosion in a premature baby or a newborn does not show any submucosal infiltration with inflammatory cells.[6, 7, 8]

Although the term "erosion" implies a loss of tissue at the surface it is now believed that such is not the case.[4, 9] Whenever it appears that areas of mucosa are missing, it is more likely an artifact arising during the preparation of the microscopic section. The base of an acquired erosion is intensely vascularized and contains many capillaries which course in every direction and give it its characteristic deep red color. It is composed histologically of three types of epithelia. First, there is columnar epithelium which is continuous with that of the cervical canal or forms isolated islands or patches (ectopia) (Fig. 3). Second, there are varying areas representing the stages of squamous

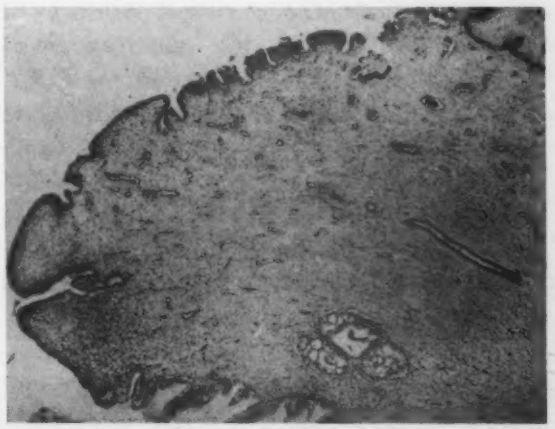

**Fig. 1. Congenital erosion of the cervix uteri in a newborn baby. (Original magnification ×25.)**

**Fig. 2.** Congenital erosion of the cervix uteri in a newborn baby. (Original magnification ×24.)

**Fig. 3.** Papillary erosion of the cervix uteri with surface of columnar epithelium and dense inflammatory reaction. (Original magnification ×150.)

prosoplasia (squamous metaplasia, epidermization) from the early appearance of subcolumnar cells to the more advanced tenuous squamous epithelium[10, 11] (Figs. 4-8). Finally, the edges on the portio are composed of squamous epithelium which seemingly sends protrusions into the eroded area. It is probable that this tissue is not an outgrowth of the squamous epithelium but is a late stage of prosoplasia. The histologic appearance of an erosion is remarkably similar to the changes which I have previously described for the normal transitional zone of the cervix uteri.[11]

The acquired erosion of postnatal life usually shows an intense submucosal infiltration with polymorphonuclear leukocytes, lymphocytes, plasma cells, and giant cells. However, it is important to note that this is not an invariable finding and an extensive inflammatory change may be lacking altogether or be minimal in amount (Fig. 8). The surface of an acquired erosion may present shallow grooves and rugae giving it a granular appearance, and when this change is pronounced it has been referred to as a "papillary erosion" (Fig. 3). When many Nabothian follicles project beneath an erosion, it has been given the name of "follicular erosion."

### Theories of origin

The oldest theory for the histogenesis of cervical erosions was proposed in 1878 by Ruge and Veit.[12] They believed that in the first stage of an erosion the surface layers of the squamous epithelium are cast off and the residual basal cells then proliferate and undergo "metaplastic" changes, resulting in a covering of columnar epithelium or the different steps of epidermization. This was an important contribution and the first to direct attention to the histology of cervical erosions. Fischel[13] in 1880 saw the appearance of columnar epithelium over the portio as a developmental anomaly dating back to embryonic life and his name has been associated with "congenital erosion." The views of Williams[14] on the origin of erosions are not well-known. He was impressed by the proliferative activity of the "cervical glands" in the lower part of the cervix and believed that in erosions the glands invade downward under the squamous epithelium.* In the course of this penetration they send out processes which burrow toward the surface, elevate the squamous epithelium, and cause it to be cast off and replaced by the columnar epithelium of the invading glands. He also felt that there is another "not improbable" way in which an erosion may be produced although he had not ob-

*On retrospection it appears that I was guilty of attempting to revive this theory in 1948, when before this Society I spoke of erosions as "adenomas of the cervix." I was not aware of Williams' work at the time.

served it himself. In this case, there is a "direct extension downwards of the epithelium of the cervical canal, a direct encroachment upon the territory of the squamous by the columnar epithelium, followed by the growth of villi, and the formation of glands."

The views of Meyer[6, 16] on the etiology, development, and healing of cervical erosions have dominated gynecologic thought for 50 years and any contradiction or divergence from these concepts calls for considerable temerity or presumption. Hamperl and his associates[9] advanced important findings which cast doubt on several of the basic tenets of Meyer's theory. However, in their conclusions, they merely state that "in general our histologic findings are in agreement with his [Meyer's] observations," but "our interpretation of Robert Meyer's classic observations is a little different."

Meyer's theory evolved on the basis of a study first conducted in his laboratory in 1910 with Adair[15] and subsequently elaborated as a result of further investigations.[16] According to this concept, an acquired erosion, in contrast to the congenital variety, is always the result of an inflammation which causes a destruction of the squamous epithelium. This excoriation is brought about by abnormal secretions which "macerate" the mucosa and result in a "true erosion." This is the first stage and is of fleeting duration. In fact, Meyer found but few histologic specimens demonstrating its occurrence. It should be noted also that in this definition a congenital erosion is taken as a completely different lesion.

The subsequent source of an erosion is then characterized by various attempts at healing, and they do not progress uniformly but the various steps overlap one another. In the first stage of healing, also known as "false healing," the eroded area is covered by columnar epithelium which is derived either from the cervical canal or from underlying "glands." The result of this process Meyer called a "pseudo-erosion," and it is comparable to the findings in an ectropion with an inflammatory reaction. It is at this point that "follicular erosions" and "papillary erosions" make their appearance. The final stage of healing, "true healing," sees the extension of squamous basal cells under the abnormal columnar epithelium and by the process of epidermization the eroded area is eventually covered with squamous epithelium.

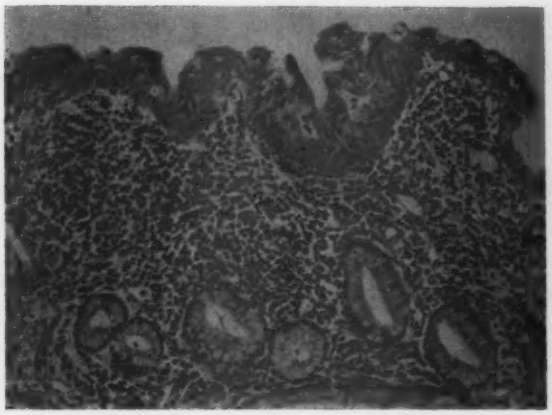

**Fig. 4.** Early prosoplasia (epidermization) in an erosion of the cervix uteri. (Original magnification ×150.)

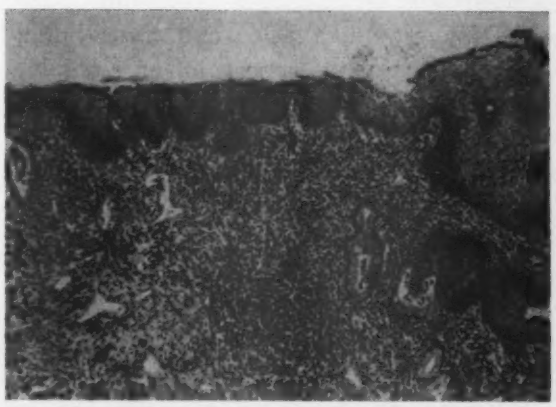

**Fig. 5.** Squamous prosoplasia (late) in an erosion of the cervix uteri during the first trimester of pregnancy. (Original magnification ×65.)

The many discussions of the subject during the past 50 years have been concerned mostly with the development of epidermization in the healing of erosions. The studies of Hamperl and his associates[9] in 1958, however, represent the first thorough attempt to repudiate several of the features of the Adair-Meyer theory. They pointed out that a loss of surface epithelium with a healing by a

**Fig. 6.** Squamous prosoplasia in an erosion of the cervix uteri. (Original magnification ×150.)

downgrowth of columnar epithelium probably does not take place and instead they advanced their own concept of an ectropion of the endocervical mucosa as an explanation for the first stage of an erosion.

### Critique of classic theory

The theory elaborated by Adair and Meyer served a valuable purpose in that it drew attention to erosions as a pathologic entity which occurs on the ectocervix not as a static lesion but as one which undergoes progressive changes from the time of its origin to the stage of complete healing. There are, however, several objections to the continued acceptance of all the details of this concept. Hamperl and his co-workers[9] have given many reasons for questioning the manner in which Meyer believed that erosions originate and they have advanced a theory of their own. It is also doubtful if the method of healing by a direct extension from adjacent squamous epithelium which undermines the surface columnar cells can be regarded as acceptable.

The features of this theory which are open to question may be considered under 4 main headings.

**1. Inflammation is the cause of acquired erosions.** Although most erosions are accompanied by an intense inflammatory reaction, it is not an invariable finding. There are typical erosions with little or no cellular infiltration of the submucosal layers. In a series of 32 cases of cervical erosions during pregnancy, of which 28 were studied from biopsy specimens, a marked infiltration with inflammatory cells was noted in 9 instances. There was only a moderate infiltration in 12 cases, while in 11 there were only occasional leukocytes, plasma cells, and histiocytes.[5]

The histologic picture of a healing erosion is remarkably similar to the normal area of the cervix which I have described as the "transitional zone,"[11] and in fact one might consider an erosion as a wide transitional zone extending from the squamous epithelium of the portio to the columnar cells of the cervical canal. Although in the adult this zone is most often accompanied by a submucosal cellular infiltration, in premature and newborn babies there is a total absence of such a reaction. This finding at least tends to show that this histologic picture in its earliest stages may occur without inflammatory changes which supervene as a secondary effect later in life.

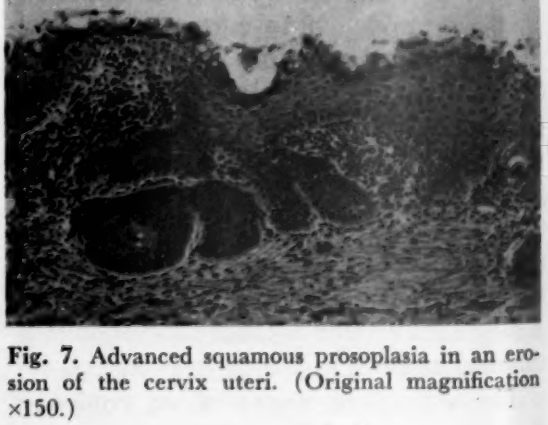

**Fig. 7.** Advanced squamous prosoplasia in an erosion of the cervix uteri. (Original magnification ×150.)

Although many acquired erosions are accompanied by inflammation, the reverse is certainly not the case, since erosions are present in only a limited number of cases of acute and chronic cervicitis. It seems most likely that inflammation with its cellular reaction, when present, is not the cause but a secondary result of a pre-existing erosion.

**2. The first stage is a "true erosion" with a loss of the surface mucosa.** The older authors believed that an "erosion" implied a superficial excoriation and Meyer taught that this is the first stage in its production, although he also believed that it is of fleeting duration and is consequently rarely seen. In fact he reported finding it only twice out of many hundreds of cases.[9, 16]

It is conceivable that only an occasional specimen showing a superficial ulceration might be found in a chronic process such as a cervical erosion. Nevertheless, it is truly remarkable that it has not been observed more frequently and has not been specifically described, especially when one considers that erosions of recent origin, as in pregnancy, have been seen by many observers both on speculum and colposcopic examination. The occurrence of such "true erosions" is questioned by Hamperl and associates,[9] and a review of the material in our laboratory fails to reveal any loss of surface mucosa which was probably not produced artificially either by the surgeon or the laboratory technician. The theory that an excoriation on a surface of squamous epithelium becomes rapidly covered by columnar epithelium in the course of a very short time and without passing through the usual prolonged stages of healing seen elsewhere in the body is incredible.

**3. The erosion resulting from a loss of squamous epithelium is covered by columnar epithelium descending from the cervical canal or arising from underlying glands.** With this concept, Meyer sought to explain the appearance of columnar epithelium over the portio and in fact this type of downgrowth also had been suggested by Williams.[14] Neither of these authors advanced adequate evidence for this remarkable method of repair of an injury to the squamous epithelium, and it has been shown experimentally that it is not the usual course of events. Both Hamperl and associates[9] and Glatthaar[17] have demonstrated that when an area of squamous epithelium near the external os is removed by scraping off with a sharp instrument the defect is healed not

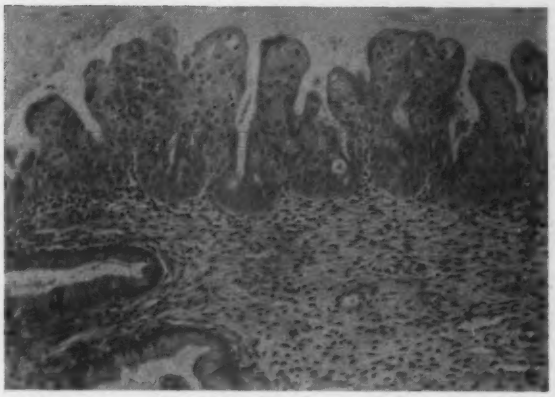

**Fig. 8.** Squamous prosoplasia in an erosion of the cervix uteri during the early puerperium. Note minimal inflammatory reaction. (Original magnification ×150.)

by columnar epithelium but by an epithelization by the adjacent squamous epithelium. The same holds true following treatment with the cautery which destroys the ectopic columnar epithelium and allows the squamous cells to cover the traumatized region.

**4. A pseudo-erosion with a base of columnar epithelium is healed when squamous epithelium infiltrates beneath the columnar cells (epidermization) and eventually covers the eroded area.** The process of epidermization (squamous metaplasia, prosoplasia) has been discussed for a long time and objections frequently have been raised to Meyer's theory that the process results by an extension of basal cells from adjacent squamous epithelium which undermine the columnar cells and eventually displace them. It is important to note exactly what this entails. It is not a simple epithelization to cover a denuded area but a complicated process during which new cells appear under the columnar cells, develop in close relationship with them, and eventually destroy them while they themselves differentiate into a stratified squamous epithelium.

There are several observations which render this theory invalid. First, it assumes that the process represents a horizontal progress of basal cells extending from normal squamous epithelium to underlie the columnar cells and dislodge them. In other words, under certain conditions, these basal cells must

acquire invasive properties akin to cancerous tissue. However, the appearance of well-preserved columnar units above several rows of basal cells is against the concept of a destructive invasion which would necessarily disrupt the nutrition of the surface elements. Second, there is no experimental evidence for such an extension of basal squamous cells. However, in the rat or mouse vagina, a similar transformation of columnar into squamous epithelium occurs cyclically during the estrous cycle, but in this case the order of growth is vertical and not in a horizontal direction. Third, a clear-cut line of demarcation between normal squamous epithelium and the atypical multilayered area seen in the cervical transitional zone is against the concept that one is an extension of the other. Fourth, the basal cells sometimes contain mucin, a characteristic of columnar, not squamous, epithelium. Fifth, epidermization (prosoplasia) often occurs in areas of the cervix where there is no adjacent squamous epithelium, for example, in mucous polyps and deep in individual clefts. It does not seem reasonable to assume that a special mechanism for epidermization is brought into play only in the presence of erosions and not under other physiologic and pathologic conditions.

Another possibility given by Meyer is that epidermization may occur from rests of squamous epithelium which have remained beneath the columnar cells and eventually become active. The question of embryonic remnants is dealt with in a subsequent section.

### First stage or formation of erosions

The last word on the question of cervical erosions certainly has not been given but at the present it seems that their histogenesis requires the consideration of two distinct phases or stages. The first deals with the formation and the second with the healing of erosions.

There is little doubt that the earliest stage of an erosion is present when an area in the region of the external os becomes covered with columnar instead of squamous epithelium. This view has been recognized by all the older authors who sought to explain its occurrence on the basis of destruction of the squamous mucosa and its replacement by either a metaplastic process or a downgrowth of columnar cells from the cervical canal. Although this explanation is adequate for many erosions, it is not always sufficient since in numerous instances there is not merely an alteration in the surface covering but the eroded area is composed of the whole depth of the mucosa and includes clefts and tunnels (glands).

Since there are valid reasons for abandoning the prevalent theories accounting for the downward migration of the columnar epithelium by an active growth along the surface, it is essential to seek other explanations for the first stage of an acquired erosion. There are three possibilities which demand consideration. First, the congenital erosion. Second, the ectropion or eversion resulting from cervical injuries. Third, a prolapse of the cervical mucosa due to changes in the volume of the cervix as described by Hamperl, Kaufmann, Schneppenheim, and Ober.[18-20]

A congenital erosion is a frequent finding not only in newborn babies but in children up to the age of 5 years, and it is also found in premature babies. In my own studies of autopsy material, I found a congenital erosion in 12 out of 29 specimens of uteri from newborn term babies, in 8 out of 14 premature babies of from 26 to 32 weeks of intrauterine life, and in 23 out of 34 infants and children from 2 weeks to 5 years of age. The incidence seems to diminish after this age since an erosion was only found twice in 8 specimens from children of 5 to 14 years of age but the numbers are too few for a final evaluation.

A number of explanations have been advanced for the production of a congenital erosion; for example, it has been suggested that it results from endocrine changes or a prolapse of the mucosa such as occurs in the maternal cervix in pregnancy. However, the fact that this lesion is found as early as the twenty-sixth week of intrauterine life

is against such explanations. The tremendous proliferation of the fetal cervical mucosa due to endocrine stimulation is not apparent until the last weeks of pregnancy. It seems more likely that it is a developmental anomaly associated with the differentiation of the epithelium of the cervical canal and the portio which probably have a common entodermal origin.[21]

It is not known how many congenital erosions persist until maturity, but it is generally accepted that a certain proportion do so and one estimate places the incidence of congenital erosions in young adults as high as 30 per cent.[22] In any case, it may be assumed that these erosions heal by a process of epidermization and once this has taken place the histologic picture becomes identical with the acquired erosion in the healing stage. From this standpoint, it is logical to accept the congenital erosion not as a separate entity but as the progenitor or first stage of the so-called acquired erosion.

The second explanation for the appearance of cervical mucosa over the portio is the ectropion or eversion that occurs as a result of lacerations of the cervix during labor. It is a generally accepted pathologic process and should be recognized also as a first stage in the acquired erosion and not as a distinct lesion which demands a separate classification.

The third explanation is also based on the concept of a prolapse of the cervical mucosa. However, in these instances it takes place even in the absence of lacerations when there is a deformity of the cervix due to a marked increase in its volume. It is a particularly satisfactory explanation for the occurrence of erosions during the course of gestation. This concept has been advanced by Hamperl, Kaufmann, and their associates[18-20] on the basis of the examination of 853 unselected specimens of uteri. The actual occurrence of an eversion or ectropion was demonstrated by the finding that the length of the cervical mucosa covered by columnar epithelium is a constant and whenever it moves downward with the resultant appearance of columnar epithelium on the portio, the upper limits (junction of cervical epithelium and endometrium) also are displaced to a lower level.

On the basis of this evidence the first stage of an erosion (or pseudo-erosion) of the cervix may be said to occur when an area in the region of the external os is found covered with columnar epithelium or it is composed of all the structures of the mucosa of the cervical canal. This condition is met when there is a persistent congenital erosion or there has been an eversion of the mucosa due either to cervical injury or to a change in the volume of the cervix which leads to a prolapse of the mucosa.

### Second stage or healing of erosions

An erosion is healed when the columnar cells are disposed of and the affected surface is covered with squamous epithelium. This is accomplished by the process known under various terms such as squamous metaplasia, epidermization, and squamous prosoplasia. The steps which lead to the end result are well-known to all gynecologic pathologists, and in fact many of the bizarre histologic pictures they may lead to are at times confused with carcinoma or carcinoma in situ. The earliest stage is characterized by the appearance of small cuboidal cells under the columnar epithelium. They have been given many names such as subcolumnar cells, basal cells, reserve cells, indifferent cells, and infraepithelial cells. These primary cells are immature bipotent units that can evolve into either columnar or squamous epithelium. When epidermization takes place, they proliferate until they form several layers and they may persist covered with normal columnar epithelium for long periods of time. Eventually, however, the columnar cells are cast off and the original subcolumnar cells proliferate and gradually differentiate until they form a mucosa identical with normal epithelium. The steps which lead to this end result do not occur as a uniform change but at varying rates so that the different stages may be seen in a single erosion.

The origin of the subcolumnar cells is the basis of this whole problem and several explanations have been advanced. As indicated

in the previous section, there is reason to question the validity of Meyer's concept that they are basal cells from adjoining squamous epithelium which have burrowed and extended under the columnar epithelium. A number of other theories also have been advanced, chiefly that the subcolumnar cells are of embryonic origin or the result of a metaplastic change, and again we are faced with objections to their unreserved acceptance.

The possibility that epidermization is accomplished by a reawakening of embryonic cell rests which have remained dormant for a long time has had many adherents. Meyer thought that primitive entodermal cells with the potency of developing into squamous elements could be present in columnar epithelium. Eichholz[22] thought that embryonic undifferentiated cells could persist as inclusions, and the more recent concept of the "reserve cell" is based on the same line of thought. However, there are many objections to the acceptance of these views. First, the subcolumnar or "reserve" cells which develop into squamous epithelium have been found in as many as 95 per cent of all cervices at all ages. This high incidence surely points to some normal constituent of the tissue rather than to an abnormal cellular inclusion. Second, there is no assurance that such cells, if embryonic, would always lead to squamous epithelium and not to some other related tissue. Third, it is difficult to explain how undifferentiated embryonic rests make their appearance as an additional characteristic of a new growth such as a mucous polyp. Fourth, epidermization frequently recurs, for instance, in succeeding pregnancies, in spite of previous extensive cauterization which presumably would destroy any preexisting primitive inclusions. Finally, it seems absurd that cellular remnants could persist for 40 to 60 years, even into old age, and then become reactivated.

The term "squamous metaplasia" is widely used, but this is incorrect if one adheres rigidly to the classic descriptions of the two types of metaplasia given us by the older school of German pathologists. The process actually is quite different from either "direct" or "indirect" metaplasia and in persisting with the usage of the term either we are guilty of ignorance as to the nature of the pathologic changes concerned or we are given to slipshod habits in the application of correct terminology. Robert Meyer no doubt was adhering to these clear-cut definitions when he emphasized that in all his experience he had never seen an instance of metaplasia in the cervix uteri.

The "direct" type of metaplasia as described by Virchow is no longer accepted as a possibility. It referred to the direct conversion of *mature* columnar cells into squamous cells. The term "indirect regenerative squamous metaplasia," on the other hand, was introduced by Fisher-Wasels[23] to describe a series of changes, possibly of a reparative nature, which are attained as the final phase of a destructive lesion. Kaufmann,[24] Oeri,[25] and Ruge[26] offered it as an explanation for the epidermization found in the cervix, and the term still is found in contemporary German literature.

Indirect regenerative squamous metaplasia occurs only as the result of a profound injury to the mucosa and the conditions for its production are not found in the common epidermization of the cervix so that it must be questioned as the likely explanation. In this process, the mucosa first suffers a local injury which leads to atrophy, degeneration, and necrosis. Some cells, however, survive and divide by mitosis to become differentiated into squamous epithelium. They are seen as small clusters or islands of well-stained polygonal cells, and this multifocal origin is an outstanding characteristic of its early stage. It accompanies chronic inflammation, atrophy, trauma, excessive and prolonged hormonal stimulation, and vitamin A deficiency. It has been described in many tissues and organs, and in the endometrium it frequently has been seen in hyperplasia of the endometrium, adenocarcinoma, and endometritis. It may occur in the cervix, for instance in certain adenoacanthomas, but it certainly is not the process which leads to epidermization in the healing of erosions.

In seeking an explanation for the manner in which epidermization of the cervix takes place and consequently the healing of erosions, it is essential to meet certain requirements. It must be a process which can occur at a distance from squamous epithelium, in a newgrowth such as a mucous polyp, and in areas which previously were destroyed by cauterization. It must not be dependent on pathologic lesions such as inflammation, degeneration, atrophy, or necrosis. It must also be responsive to endocrine stimulation since it can be enhanced by the administration of estrogenic hormone.[27] Finally, the basic unit responsible for this change, the subcolumnar cell (basal cell, reserve cell) must be dependent on a normal constituent of the mucosa.

The theory of "squamous prosoplasia" which I[10] presented before this Society in 1954 fulfills the requirements and avoids the objections to the other theories which have been mentioned. This concept assumes that the subcolumnar basal cells are derived directly from the columnar cells and have the potentiality of developing into either columnar or squamous epithelium. This bipotency is possible because they have a common embryonic origin, probably from the entodermal urogenital sinus,[21] and the same changes can be seen in related tissues such as the Bartholin glands and urethra.[10]

The process of prosoplasia is an orderly, progressive development. In contrast to the other theoretical explanations, it implies a physiologic transformation and it is dependent neither on mythical embryonic cells nor on a pathologic stimulus such as the cellular destruction which precedes indirect metaplasia. The fact that it is a constantly recurring change is amply demonstrated by the part it plays in the transitional zone of probably all cervices and at all age periods from prenatal life to old age.[11]

This prosoplastic redifferentiation or rejuvenescence of columnar epithelial cells has considerable support from experimental observations. A dedifferentiation is the usual sequence in successive tissue cultures of human columnar cells from the cervical mucosa. The vagina and cervix of the spayed rat undergoes a pronounced atrophy until it is composed of only one or two layers of small cuboidal cells. I have found repeatedly that by stimulation with varying amounts of estrogen and progesterone this mucosa may differentiate into stratified squamous epithelium, or a multilayered mucosa composed almost entirely of mucus-secreting cells, or combinations of the two. The normal changes of the rodent vagina also present either columnar cells or squamous elements according to the stage of the estrous cycle. These two observations clearly demonstrate the bipotency of the cells of the rat cervix and vagina and there is good reason to believe that the same holds true in women.

**Summary**

There are many objections to the continued acceptance of the prevalent theories for the histogenesis of erosions or pseudoerosions of the cervix uteri. This aspect of the subject is discussed in detail.

An acquired erosion may be defined as having two main phases. In the first, or the period of formation, an area of the external os becomes covered with a single layer of columnar cells or with all the constituents normally found in the cervical columnar mucosa. The second, or healing stage, is characterized by epidermization, a process which leads to a casting off of the columnar cells and the surface becomes covered with squamous epithelium.

The columnar cells find their way onto the portio as the result of either a congenital erosion or an eversion or prolapse of the endocervical mucosa.

The process of epidermization is not satisfactorily explained by an ingrowth of basal cells from adjoining squamous epithelium or by the persistence of embryonic cell rests, or by direct or indirect metaplasia. On the other hand, the change defined as squamous prosoplasia, which assumes that the bipotent subcolumnar cells which eventuate into squamous epithelium are derived from the columnar cells, seems to have none of the objections advanced against other theories.

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## Discussion

Dr. Daniel G. Morton, Los Angeles, California. There seems to be little disagreement regarding the nature of the so-called congenital cervical erosion. All agree that the visible red area about the external os is covered by columnar epithelium and is in fact exposed endocervix. The thought that the squamocolumnar junction has simply become localized more externally than normally seems to be acceptable as a developmental or congenital vagary. Dr. Fluhmann makes the interesting observation that such lesions are more common in young children than in young adults, suggesting that nature eventually covers over the area spontaneously in many instances. He suggests that the rising tide of estrogen at the menarche may be responsible for the epithelization with squamous cells which takes place at about this time. Ordinarily, there is no inflammatory element associated with congenital erosion and it requires no treatment.

There is no confusion regarding the nature of the red circumoral area designated as "eversion" or "ectropion." Everyone accepts the explanation that such areas represent everted endocervix, and they are in fact covered by columnar epithelium. An inflammatory reaction is associated with these eversions in most instances. The lateral lacerations consequent upon childbearing constitute the most usual etiological factor. Some investigators have suggested that hypertrophy or distention of the cervix from whatever cause (e.g., pregnancy) may also cause "prolapse" of the mucosa or eversion and therefore play a role in the production of such lesions.

In contrast to what has been said regarding congenital erosion and eversion, considerable confusion still exists with respect to the precise nature of the acquired cervical erosion. As a matter of interest, I consulted a dozen modern textbooks for definitions. Nine of the 12 authors described the acquired erosion as a red circumoral area covered by columnar epithelium, while 3 authors spoke of the erosion as representing an area denuded of its epithelial surface. Two of the latter mentioned scattered areas of columnar epithelium on the otherwise denuded area in some cases. In 5 instances, no description of the development of the erosion was ventured. Several

of the authors accepted the explanation given by Robert Meyer. Several others spoke of maceration and sloughing of the squamous covering of the circumoral area due to alkaline discharge.

With respect to the healing of the acquired erosion, 5 of the 12 authors made no definite statements. The others spoke of growth of squamous epithelium from the margins of the affected area toward the original external os, some in the manner described by Robert Meyer, and some referred to superficial covering of the area by squamous epithelium, not an undermining. Jeffcoate states that cauterization is effective treatment of such lesions because it destroys the columnar cell covering, leaving a fresh denuded area upon which the squamous epithelium grows in from the margins.

The total of these observations is confusion, i.e., no agreement as to what it is, how it got there, or how nature combats it. The truth of the matter is that the right kind of material for the study of the problem is hard to come by. To determine the proper relationship of the endocervical elements to the portio vaginalis and its usual epidermoid covering, one would require generous blocks of tissue extending well above and below the affected area, carefully oriented, and in order to determine accurately the process of repair, one would require frequently repeated biopsies of this type, an almost impossible task.

Dr. Fluhmann has given us an excellent discussion of the deficiencies of the various theories regarding acquired cervical erosion. He has suggested that the acquired erosion presents a covering of endocervical columnar cells which have found their way onto the portio as a result of either a congenital erosion or an eversion or prolapse of the endocervical mucosa. He appears not to accept complete denudation of the surface as responsible for any of the so-called erosions. In this respect I think that he is wrong, though I believe that he is correct in perhaps the majority of the cases. Unfortunately, I have no body of material to present in support of my opinion but I recall the observation of a missing epithelial cover in many sections of cervices which I have seen. I would doubt that the explanation could be surgical manipulation.

Of great interest, too, are Dr. Fluhmann's ideas regarding the process of re-epithelization of the erosion by epidermoid cells. He visualizes a dedifferentiation of the columnar cells with regrowth and redifferentiation into epidermoid cells. That epidermization of subcolumnar, reserve cells is very often associated with cervicitis and erosion and is often a widespread process is certainly a fact. It is also plausible that proliferation of this layer of cells may lift off the columnar cells and cause them to disappear. We can all agree with him, I feel sure, that this layer of epidermoid cells cannot be accounted for by an ingrowth of basal cells from the adjoining squamous epithelium or by the existence of nests of squamous cells beneath the mucosa. However, I find myself in need of further conviction with respect to two features of Dr. Fluhmann's explanation of the process, namely, the origin of the reserve cell layer from which the epidermoid cells derive by a process of dedifferentiation of the columnar cells, and, second, his failure to consider a covering proliferation of squamous cells from the periphery as an important feature of the mechanism of re-epithelization. In regard to the former, I must say that I do not have a good substitute origin to suggest but have always thought of the reserve cells as arising from the stroma, or, at least, in the stroma just beneath the columnar lining and possibly being of specialized origin.

It seems simpler and more acceptable to me to account for re-epithelization, for the most part, as the growth of squamous cells from the periphery of the erosion. Since re-epithelization occurs so readily after cauterization of the surface—i.e., destruction of whatever bits of epithelium there may be there, either of columnar or epidermoid origin—I cannot conceive of the mechanism suggested by Dr. Fluhmann as accounting for the re-covering of the surface by squamous cells. We certainly know that epithelization of raw surfaces from the margins of intact squamous epithelium is a property possessed by the epithelium of both the skin and the vagina as, for example, in the case of the creation of an artificial vagina. Why should we not expect similar behavior from the squamous epithelium of the portio vaginalis of the cervix?

Dr. Ronald R. Greene, Chicago, Illinois. I certainly think what Dr. Fluhmann has told us today is basically correct, but I am bothered by his semantics. I cannot differentiate indirect metaplasia and direct metaplasia, but I think we agree about so-called erosions. I have had the opportunity of examining student nurses when they enter nurses training and have kept statistics on our findings. About 70 per cent of these young women are virgins, and of the whole group some-

where about 25 per cent have so-called congenital erosions. These have not been biopsied but I am sure they are not true erosions in the pathologic sense. "Erosion" should mean loss of surface epithelium and here we are actually talking about a condition in which the cervical mucosa extends out onto the ectocervix.

DR. C. LEE BUXTON, New Haven, Connecticut. Dr. Greene's remarks prompt me to call to your attention a theory of the etiology for ectropion or erosion which has been elaborated mostly by Ober in Kaufmann's Clinic in Cologne—that is the possible hormonal etiology of these conditions. This theory proposes that columnar epithelium in the cervix has a marked tendency to respond to estrogen, more so than the squamous epithelium of the vagina and, therefore, during active estrogen secretion columnar epithelium protrudes from the cervical canal and thus produces ectropion. This theory has been at least partially proved experimentally in castrates given estrogen in whom this type of ectropion has been produced. It may explain the fact that in the postpartum patient what we frequently consider to be a bilateral laceration of the cervix may not be such at all but may be protrusion of the columnar epithelium of the cervical canal, thus presenting the appearance of being more extensive on the anterior and posterior surfaces of the cervix than at 3 and 9 o'clock.

DR. FLUHMANN (Closing). The name "erosion" is basically incorrect but it has been in common usage for so long that it would be difficult to introduce a new term. It is important, however, to distinguish between an erosion and an ulceration and the early writers on the subject were careful to do so. Possibly the word "pseudo-erosion," suggested by Robert Meyer, would be more acceptable.

It is surprising that Dr. Morton believes that a loss of the surface epithelium does occur in some erosions. As pointed out in my paper, I feel that most of the evidence is against this belief.

Dr. Greene is probably quite correct in stating that some of the divergent views regarding cervical metaplasia arise more because of semantic difficulties than because of clear-cut differences in the pathologic findings. I have been hesitant to introduce the term "squamous prosoplasia" but there seemed to be a need for a name that would differentiate this process from the classic direct and indirect metaplasia seen in pathologic lesions.

Although I do not know if it would be possible to produce an erosion experimentally by the administration of hormones, as Dr. Buxton suggests, there is no doubt that the erosions seen during pregnancy result directly from the endocrine changes induced in the cervical mucosa at this time.

# Absence of classical homologous tissue reactions following injections of embryonic tissues

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PREVIOUS studies showed that it was always possible, by ordinary microscopic methods, to distinguish between autologous and homologous transplants of musculofascial tissues in nonpregnant adult rabbits. Both types of transplants underwent similar sequences of degeneration, absorption, and organization with exception of the homologous transplant in which a sterile inflammation was superimposed upon the events of repair, especially within the musculofascial zone. This pattern of tissue reaction was a reproducible biologic phenomenon and served to distinguish the tissues of one individual from those of another of the same species and strain.[1]

Studies in normal pregnant rabbits revealed that it was not possible to distinguish between autologous and homologous musculofascial transplants. Instead, both types of transplants underwent similar patterns of tissue reaction identical to the reaction of the animal to transplants of its own tissues.[2]

Other studies in pseudopregnant hosts demonstrated that regardless of the hormonal changes and the associated hyperplasia within the genital tract of the recipient, the harmonious host-homograft interaction so characteristic of normal pregnancy did not appear.[3]

In view of the capacity of embryonic tissue extracts to accelerate mammalian cellular growth in vitro as well as in vivo[4] and the known proliferative potential of rabbit embryonic tissues following transplantation,[5] it seemed reasonable to assume that developing embryos in utero may elaborate growth-regulating substances which may cause the pregnant host to react to homografts in a manner identical to transplants of its own tissues. With these in mind, the following experiments were designed to characterize the homologous tissue reaction in adult hosts harboring transplants of proliferating embryonic tissues.

## Method

Fourteen pregnant New Zealand rabbits between 14 and 16 days of gestation were operated on under pentobarbital anesthesia and with sterile precautions. In individual experiments, the embryos, averaging 7 to 14 per litter, were removed from the herniated amniotic sacs following small incisions in the antimesenteric border of the uterus. The embryos were recovered in a 20 ml. test tube and washed six times in normal saline solution (Fig. 1, typical embryo). Following the washings, 1 to 2 ml. of normal saline solution was added to the tube and the embryos were then gently crushed with a Potter's rod. After maceration, the embryonic tissues were

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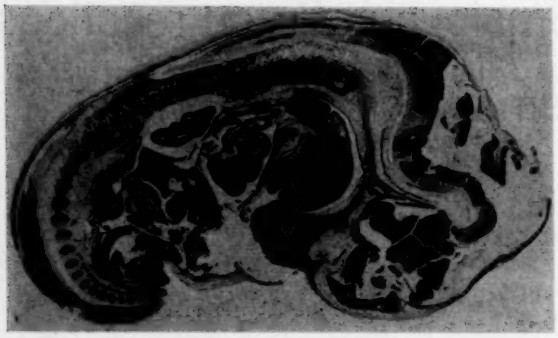

**Fig. 1.** *Low-power photomicrograph of a rabbit embryo between 14 and 16 days of gestation.* Note the degree of organ differentiation at this stage and the well-developed vascular system. The circulating blood cells at this time consist essentially of nucleated erythrocytes. Embryonic tissues of this age possess conditioning mechanisms comparable to adult circulating leukocytes following injection into adult hosts. (Hematoxylin and eosin. ×5; reduced ⅜.)

poured into a 5 ml. syringe already fitted with a 16 gauge needle.

In the meantime, an adult male or female rabbit was prepared to receive the embryonic tissues. The back of the recipient was shaved and the skin prepared with aseptic technique. Under dermal procaine anesthesia and with sterile precautions, an incision was made perpendicular to the vertebral column in the left lumbar region. The panniculus carnosus and superficial fascia were divided so as to allow direct vision of the fascia overlying the erector spinae. The embryonic tissue mince was then injected into the erector spinae. The puncture site in the fascia and the superficial fascia were closed with nylon suture. The panniculus carnosus and skin were closed, respectively, with plain catgut and nylon. Collodion was sprayed over the wound.

Six days following the intramuscular injection of embryonic tissues, each pretreated host received a musculofascial transplant, 15 mm. in length and breadth and 8 mm. in thickness, resected from the erector spinae of a random adult donor under similar sterile precautions. The recipient site was prepared by resecting and discarding a cuboid block of muscle of similar dimensions from the erector spinae in the right lumbar region. The homologous transplant from the random donor was then embedded and sutured into place with 4 monofilament nylon sutures which drew the fascia of the transplant into close approximation with the fascia of the recipient site. The wound was closed in the aforementioned manner.

Two weeks later, the pretreated host was killed by injection of procaine solution into the cisterna magna. The skin was removed from the back and a ventral incision made in the abdomen. The body was then immersed in 10 per cent formalin. After 4 days of fixation, the embryonic and musculofascial transplantation sites were cut in serial thin blocks in a direction perpendicular to the muscle fascia. The serial blocks of tissue, including the residual transplant and all tissues adjacent to it, were prepared for microscopic study in sections stained with hematoxylin and eosin.

Several animals were killed 6 days after injection of embryonic tissues and the transplantation sites were similarly prepared for microscopic study.

## Results

Gross examination of the homologous musculofascial transplants following fixation showed that they were well-healed in place. A bursal sac had formed as usual over the transplant. A delicate red-streaked translucent pannus of granulation tissue was noted overlying the white fascia of the transplant.

Gross examination of the embryonic tissue injection sites 6 days and 20 days of age following serial section showed irregular, soft, dark areas within the skeletal muscle of the host's erector spinae. The areas were larger in size in the 6 day injection sites.

Microscopic study of all homologous musculofascial transplants revealed them to be securely healed in place. A vascularized pannus of host's granulation tissues had grown over the fascia of the transplant. From the pannus, vascular channels had grown down through the fascia to terminate in a peculiar angiomatous ramification directly beneath the fascia. Mechanisms of autolysis and absorption, normally operative within musculofascial transplant, following transplantation were impaired so that muscle bundles re-

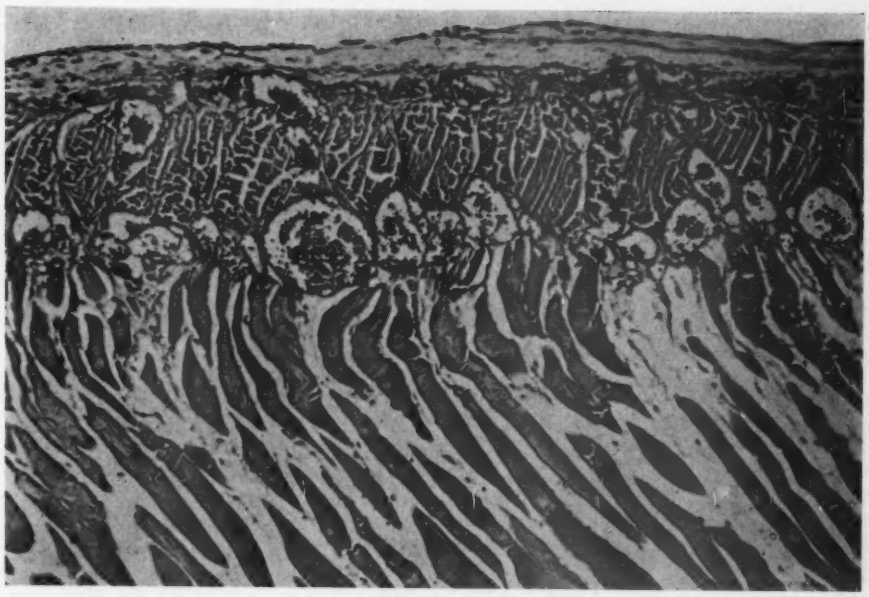

**Fig. 2.** ***Medium-power photomicrograph of a part of a homologous transplant 2 weeks after transplantation into an adult host pretreated with pooled embryonic tissues.*** The space above the tissue is the bursa. A vascularized pannus of host granulation tissues has grown over the fascia of the transplant. Angiomatous channels, containing erythrocytes of the host, have developed beneath the fascia. Mechanisms of autolysis and absorption of skeletal muscle within the musculofascial zone have been delayed. Note the absence of classical homologous tissue reactions as illustrated in Figs. 6 and 7. There are no apparent differences in the postleukocytic transfusion homologous reaction and the postembryonic tissue injection homologous reaction. Compare Figs. 8 and 2. (Hematoxylin and eosin. ×200; reduced ⅓.)

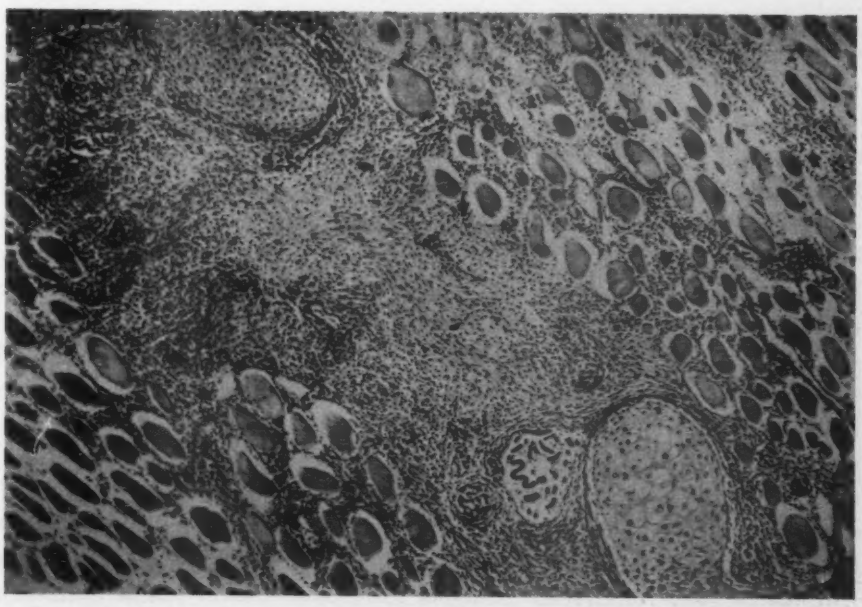

**Fig. 3.** ***Medium-power photomicrograph of rabbit embryonic tissues 6 days after injection into the adult host.*** The injection site consists of fragments of cartilage with dermal and glandular elements embedded in a proliferating, well-vascularized matrix of embryonic skeletal muscle. Skeletal muscle fibers of the host are noted at the upper right and lower left margins of the illustration. (Hematoxylin and eosin. ×200; reduced ⅓.)

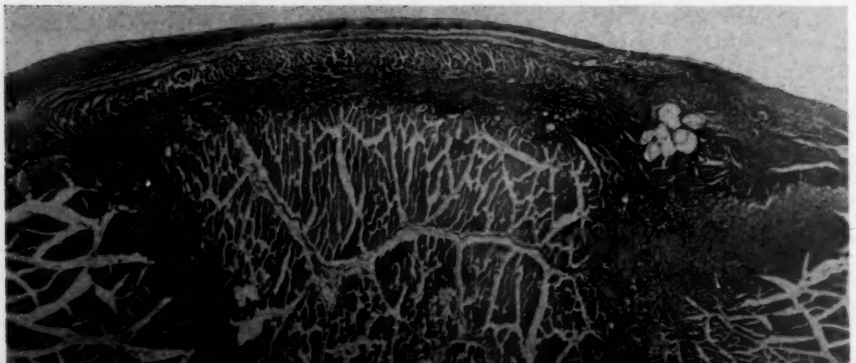

**Fig. 4.** ***Low-power photomicrograph of a homologous musculofascial transplant 2 weeks after implantation in the normal pregnant host.*** The empty space above the tissues is the bursal sac. The floor of the bursal space consists of a richly vascularized pannus of connective tissues originating from the pregnant host. The fascia of the transplant is permeated by blood vessels from the overlying pannus. These terminate in the broad musculofascial zone which consists of proliferating fibroblasts and atypical myocytes embedded in a well-developed, dense collagenous matrix. Vascularized connective tissues are replacing the region vacated by the degenerating muscle of the lateral and inferior margins of the transplant. (Hematoxylin and eosin. ×20; reduced ⅓.)

mained in almost their original relationship to the fascia. In spite of the rich blood supply, inflammation, usually a conspicuous component in the reaction to homologous tissues, did not occur (Fig. 2).

Microscopic study of the embryonic tissue sites at 6 days after injection, showed fragments of cartilage, dermal elements and glandular tissues embedded in a well-vascularized, proliferating matrix of embryonic skeletal muscle (Fig. 3). Examination of the embryonic tissue sites at 20 days after injection showed that proliferating activities had slowed considerably. Cartilage was beginning to show deterioration. All dermal and glandular elements had disappeared. The matrix of embryonic skeletal muscle was being replaced by dense scar tissue from the host. Scattered small foci of plasma cells and lymphocytes were observed.

## Comment

The use of musculofascial tissues in the experimental study of the failure of homografting afforded several advantages. First, musculofascial tissues were abundant and readily accessible. Second, these tissues could be handled with sterile precautions and implanted in protected locales which could be critically studied by usual microscopic methods. Last, tissues of the transplant and those of the host remained distinct so that interactions between transplant and host could be accurately interpreted.

The freshly resected musculofascial transplant was composed of two distinctly different types of tissues. The attached fascia consisted of heavy bundles of collagen between which were dispersed fibrocytes. From the fascia delicate strands of collagen passed to ensheath the skeletal muscle fibers in secure fashion. The junction between fascia and muscle was merely a potential space. However, following implantation the most distinctive differences between autologous and homologous transplant-host interactions occurred within this junction.

With the passage of time following trans-

plantation, both types of autologous and homologous transplants showed similar rates of absorption so that by the fourth or fifth week very little of the transplant remained. Two weeks after transplantation was the optimal time for study.

The autologous musculofascial transplant, at 2 weeks of age, was well healed in place. A bursal space had formed over the transplant. The floor of the space consisted of a well-developed, vascularized pannus of host's granulation tissue which had grown over the fascia of the transplant. Vascular channels coursed downward from the pannus to permeate the fascia. From the level of the fascia, channels continued to ramify within the musculofascial zone. This was broad and consisted of proliferating fibrocytes and atypical myocytes embedded in a fibrillar collagenous matrix. Signs of active inflammation were minimal. The lateral and inferior margins of the transplant were slowly undergoing absorption and being replaced by formation of granulation tissue by the host. This was the pattern of tissue reaction of the animal to its own tissues (Figs. 4 and 5).

Examination of the homologous musculofascial transplant, at 2 weeks of age, showed that the pattern of degeneration, absorption, and organization was the same as that noted in the autologous transplant with the exception of a diffuse accumulation of lymphocytes derived from the host. These were situated especially within the musculofascial zone and around the host's vessels within the fascia and overlying pannus. Angiitis of the obliterative type was frequently observed within the musculofascial zone. Fibroblastic activity and collagen deposition were deterred. The lateral and inferior aspects of the transplant did not reveal further differences from similar locales about autologous transplants. The inflammatory aspects of the homologous tissue reaction served to distinguish between the tissues of individuals of the same species and strain of rabbits (Figs. 6 and 7).

Further studies showed that the classical homologous tissue reaction could be modified

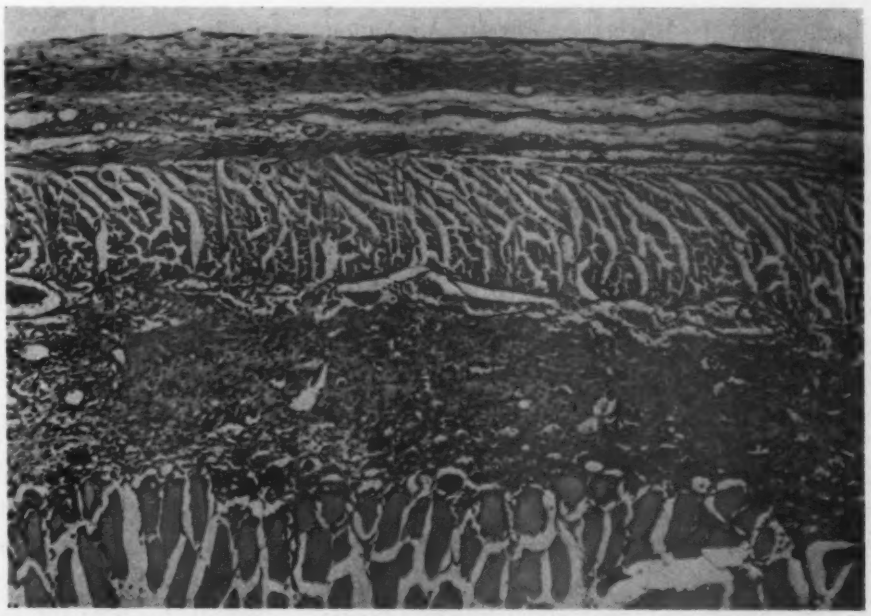

**Fig. 5.** ***Medium-power photomicrograph of a part of the homologous musculofascial transplant shown in Fig. 4.*** **The space above the tissue is a portion of the bursal space overlying the transplant. The pannus consists of well-vascularized granulation tissues produced by the pregnant host. The fascia of the transplant has been permeated by a diffuse downgrowth of vascular channels from the overlying pannus. The musculofascial zone contains a well-developed vascular plexus embedded in a dense collagenous matrix. Signs of active inflammation are minimal. This pattern of tissue reaction is identical to that observed in autologous musculofascial transplants. (Hematoxylin and eosin. ×200; reduced ⅓.)**

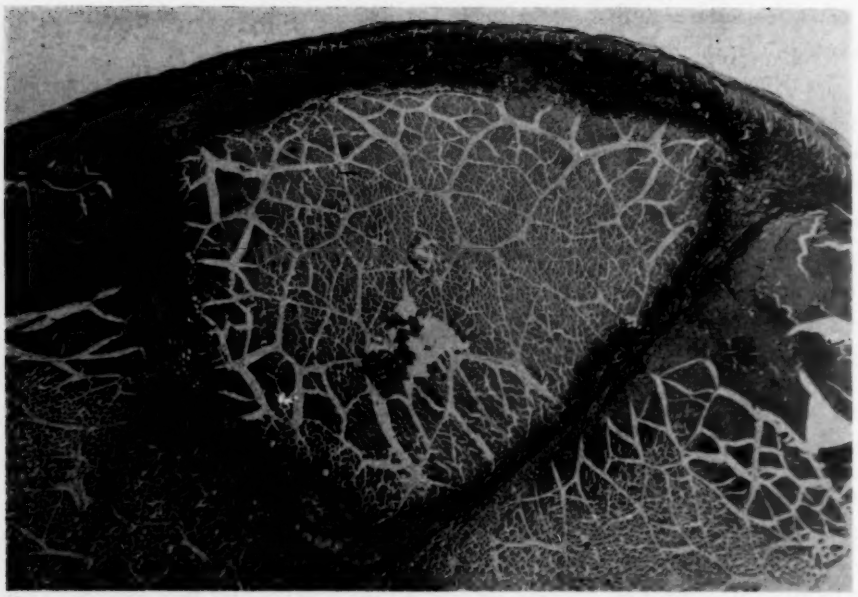

**Fig. 6.** ***Low-power photomicrograph of a homologous musculofascial transplant 2 weeks after implantation in the nonpregnant host.*** The empty space above the tissues is the bursal sac which regularly forms over musculofascial transplants. The floor of the bursal sac consists of a pannus of host's vascularized granulation tissue which has grown over the fascia of the transplant. The fascia contains many blood vessels which have originated from within the overlying pannus. The musculofascial zone is well-developed and darkly stained because of a massive infiltration of lymphocytes originating from the host. The muscle of the lateral and inferior margins of the transplant is undergoing absorption and is being replaced by proliferating granulation tissues of the host. (Hematoxylin and eosin. ×20; reduced ⅓.)

if the host had received several musculofascial transplants from the same donor prior to transplantation of the third transplant from the same donor. Examination of the last transplant showed an acute thrombotic, necrotizing reaction within the musculofascial zone.[1] This has been designated as the acute sensitizing homologous tissue reaction.

Additional studies, other than free grafting, demonstrated that modification of the classical homologous tissue reaction could be achieved by various methods of conditioning the host prior to transplantation. The most conspicuous feature of these experiments was the complete elimination of inflammation to homologous musculofascial transplants.

The failure of homologous musculofascial tissues to elicit inflammation was first observed in the study of cross-homografts in postparabiotic rabbits. Two types of tissue reaction were noted which differed only in the degree of vascular penetration by the host. Half of the homografts studied remained free of vascular penetration and were encapsulated by dense collagenous tissue. The remainder of the postparabiotic cross-homografts showed prompt vascular penetration by the host. In spite of the well-developed pattern of vascularization, absorption of skeletal muscle and stromal development were critically impaired. Angiitis, so frequently observed in the classical homologous incompatibility reaction, did not occur. The postparabiotic homologous tissue reaction was readily reproducible between parabiotic twins and seemed to bear no relation to the length of time in parabiosis prior to grafting.[6]

Since cross-circulation of blood, though transient in nature, was a dominant feature of parabiosis, further experiments were done which involved the cross-transfusion of large quantities of blood between paired rabbits. The study of cross-homografts in cross-transfused animals revealed a homologous tissue reaction identical to the vascular form noted following parabiosis. The posttransfusion homologous tissue reaction was always re-

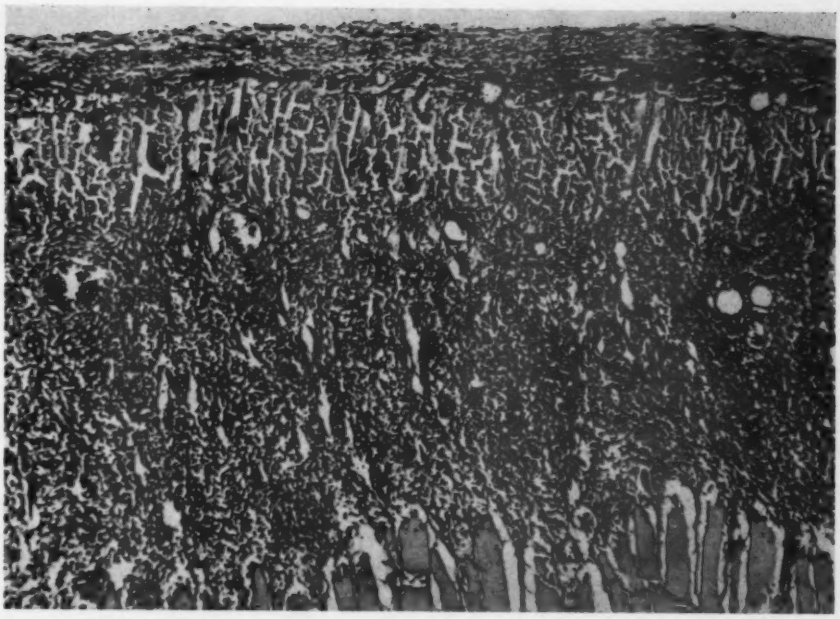

**Fig. 7.** *Medium-power photomicrograph of a part of the homologous musculofascial transplant shown in Fig. 6.* The space above the tissue is the bursa. The floor of the bursa is formed by host's granulation tissue which is well vascularized and heavily infiltrated with lymphocytes. Vascular channels pass from the pannus through the fascia to terminate in the broad musculofascial zone. This zone contains degenerating and proliferating muscle cells between which are dense infiltrations of lymphocytes and vascularized matrix of mesenchyme. (Hematoxylin and eosin. ×200; reduced ⅓.)

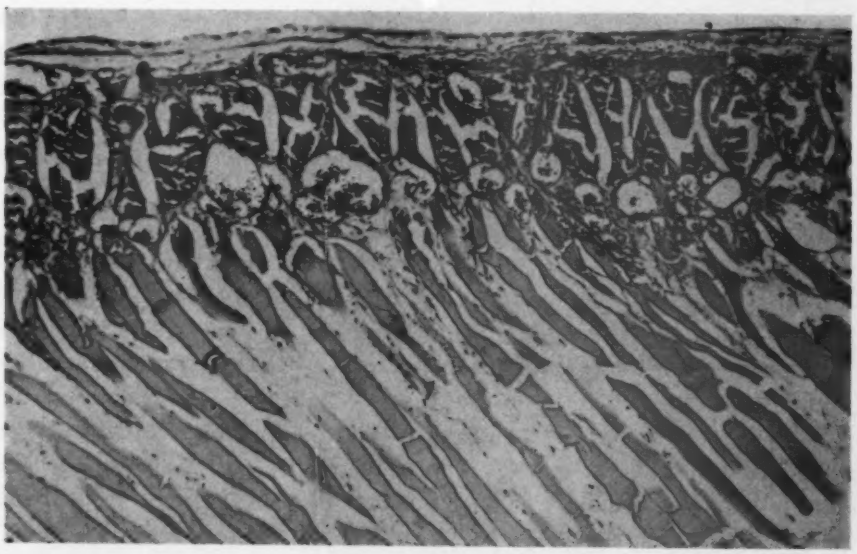

**Fig. 8.** *Medium-power photomicrograph of a part of a homologous musculofascial transplant 2 weeks after implantation in the pregnant host which had been transfused with graft donor's leukocytes prior to grafting.* The empty space above the tissue is the bursa. A well-formed pannus arising from the pregnant host remains firmly attached to the fascia of the transplant. Vessels from the pannus have terminated beneath the fascia in peculiar arrangement of patent angiomatous channels. Degeneration and absorption of muscle has been impaired as well as the sequences of stromal formation. This pattern of tissue reaction is unlike the classical homologous tissue reaction illustrated in Fig. 6 and also different from the homologous tissue reaction of the nontreated normal pregnant host as illustrated in Fig. 4. (Hematoxylin and eosin. ×200; reduced ⅓.)

producible between cross-transfused animals and bore no relation to the different blood groups in rabbits.[7]

Further studies showed that the leukocytic fraction of whole blood was responsible for consistently conditioning the nonpregnant host in a manner to elicit typical posttransfusion homologous tissue reactions.[8]

Studies in pregnant hosts, pretreated with adult leukocytes prior to grafting with musculofascial tissues from the same donor, showed that the normal pregnant host was as equally reactive to the pretreatment as was the nonpregnant adult host (Fig. 8). The usual compatible homologous tissue reaction of normal pregnancy was not observed and instead a tissue reaction identical to the vascular postparabiotic and/or the postleukocytic transfusion homologous tissue reaction was noted.[9]

It was soon learned that the relationship between donor and recipient was not a specific one in terms of host-homograft interaction. Approximately one third of homologous musculofascial transplants when implanted in postparabiotic hosts from control random donors elicited a tissue reaction identical to that observed between specific donor and recipient.[10] Further confirmation of the nonspecificity of the modified homologous tissue reactions became apparent in the study of adult hosts receiving pooled blood transfusions from donors other than the donors of the transplants. In this instance, by transfusing blood from 6 donors into a single recipient prior to grafting, the percentage of posttransfusion homologous tissue reactions could be elevated to 80 per cent.[11]

The nonspecificity of the modified host-homograft interaction is also apparent from the present experiments. The injection of pooled embryonic tissues (7 to 11 embryos) into a single adult host prior to random grafting of adult tissues causes a host-homograft interaction similar to those observed in hosts receiving pooled blood transfusions prior to grafting from a random donor.

Very recent studies have demonstrated that uteroplacental massage of the entire litter of the pregnant rabbit at midgestation prior to grafting with adult musculofascial tissues can influence the maternal host–adult homograft interaction in a manner identical to the modifications previously described.[10]

Although the modified as well as the unmodified patterns of homologous tissue reactions remain unexplained, these studies form a basis for further investigations into areas of fetal-maternal incompatibility.

**Summary**

Fourteen adult rabbits each received a single intramuscular injection of pooled rabbit embryonic tissues (7 to 11 embryos per litter) prior to grafting with musculofascial tissues resected from adult random donors. In all instances, the pattern of tissue reaction in and about the homograft was identical to host-homograft interactions observed in pregnant or nonpregnant hosts pretreated with adult leukocytes prior to grafting from the same donor.

The pattern of tissue reaction was characterized by prompt vascular penetration by the host in the absence of inflammation and stromal organization. The tissue reaction was unlike that seen in normal pregnant hosts in which healing sequences involving the homograft resembled those involving autografts.

These studies have revealed the remarkably mature antigenicity of embryonic tissues at midgestation as indicated by their capacity to modify local classical homologous tissue reactions.

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## Discussion

Dr. Somers H. Sturgis, Boston, Massachusetts. The subject of Dr. Andresen's paper is unusual on this program for several reasons. First, it deals with the field of immunology and phenomena of homograft rejection with which many of us are not too familiar. Second, Dr. Andresen has distilled the result of about 9 previous experiments stretching over more than 8 years, setting the stage for the present report. The careful planning and close reasoning involved in these preliminary experiments are difficult to appreciate without reading through the original papers. I have had the privilege of time, since I was the discussant, to look up and read most of these earlier reports. Finally, this presentation is important because it brings to our attention certain basic new disciplines that bear on a major puzzle underlying all obstetrics. The puzzle is this: Since the fetus derives one half its chromosomes from the father, why does not every mother call upon her immunologic defenses to reject such a monster as she would reject any other homografted tissue with different chromosomal pattern from her own? According to strict basic immunologic principles, the only fetus that would have the right to survive would be that born to parents who were identical twins. It is to the clarification of this puzzle that Dr. Andresen as well as many others has dedicated his research.

The mechanisms involved in homograft rejection are only slowly becoming untangled. Skin grafts have been used most extensively because changes in these can be appreciated visually from day to day and biopsies can be taken, but only recently have we been provided with sequential morphologic changes associated with microscopic data.[1, 2] Although rejection phenomena have been studied in bone, cornea, ovary, kidney, and lymph nodes, Dr. Andresen alone as far as I am aware has utilized the musculofascial block graft. His observations, then, cannot be directly compared with or contrasted to those of any other worker. Each tissue apparently has its own antigenicity and evokes its own pattern of rejection. Yet, the basic principles underlie them all. Thus, the authors found that their type of homografts were tolerated through the last 2 weeks of pregnancy, in the rabbit, just as if they were autografts. This seems to confirm the report by Heslop, Krohm, and Sparrow[3] that pregnancy in rabbits permits prolonged survival also of skin homografts. What is there in the pregnant animal that modifies or slows up rejection of skin and musculofascial grafts? Why is it that trophoblastic cells may be recovered in numbers from circulation and may lodge, survive, and live in the lungs, but rapidly disappear or are "rejected" once pregnancy is terminated? In the authors' report, it is noted that if pregnancy was terminated within 4 days of grafting, the homotransplants were promptly rejected. Can all this be a reflection of some change in hormonal production, or is it something that the embryos themselves contribute to immune mechanisms?

Two hypotheses concerning the nonrejection of the fetus by the mother are worth exploring:

First, it has been stated that the fetus is immunologically immature and incapable of producing antibodies. This obviously has no bearing on prolonged survival of skin or other homografts in the pregnant state.

Second, Heslop and associates point out the possibility that an increased production of adrenal cortisone is responsible for the modified homologous tissue reaction seen in pregnancy. There is little doubt that the improvement of patients with Addison's disease and of some with rheumatoid arthritis is thus explained. The maternal adrenals are known to produce large increments of corticoids in pregnancy. Most of these substances are in the "bound" form and only a moderate increase is reflected in the urine. Besides the maternal adrenals, the fetus's adrenals and the placenta also produce cortisone.

Indeed, large amounts of ACTH can be extracted from the placenta itself.[4]

The increased production of cortisone from all sources in pregnancy may be a biologic mechanism to combat the rejection of the fetus by the mother, as well as that of trophoblastic cells that escape into the general maternal circulation.

Dr. Andresen suggests that the capacity of his pooled embryo minces to modify the classical homologous tissue reaction in the pregnant host and indicates that these minces have a remarkably mature antigenicity. I am not able fully to accept this point of view. Although I assume that the placentas, with their ACTH and cortisone, were not included in the embryo minces, it seems more reasonable to me to ascribe the less violent rejection of musculofascial homografts after injection of these minces to the added cortisone that might have resided in the adrenals of the embryos minced for injection.

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DR. GORDON WATKINS DOUGLAS, New York, New York. The question of fetal-maternal tissue compatibility has a general importance to as well as a direct bearing on the field of obstetrics. It has been evident for some time that pregnancy is the most prominent exception to the general rule and that the tissues of one individual will tolerate the grafted tissues of a genetically different individual for a very limited period of time unless certain clinical and immunologic tricks are employed. The mechanisms involved in this remarkable instance of tolerance are therefore a subject of keen interest since they may contain helpful information in the field of tissue and organ transplantation. Dr. Andresen's work touches on this larger issue and for this reason I hope he will forgive me if I comment on the implications of his paper rather than on his direct observations and conclusions.

The observation that the pregnant rabbit does not exhibit the usual tissue response to an homologous musculofascial graft, but instead reacts to this transplant as if it were a graft of its own tissues, raises the question of whether this is an example of general alteration in tissue reactivity in pregnancy or whether it is due to some aspect of the experimental procedure itself. Medawar[1] noted prolonged survival of skin homografts in the pregnant rabbit at the twenty-second day but this does not apply in the case of other laboratory animals. Has Dr. Andresen attempted musculofascial homologous grafts in guinea pigs, rats, or mice? Medawar and Sparrow[2] showed that cortisone is capable of prolonging the survival of skin homografts in the guinea pig but incapable of blocking the accelerated rejection of grafts in an animal previously sensitized by grafts from the same donor. Does cortisone abolish the homologous tissue reaction to musculofascial grafts in the nonpregnant animal, and will the use of prior sensitizing grafts restore it in the pregnant animal?

It is of interest to note that Woodruff,[3] also working with pregnant rabbits, removed a fetus from one horn of the uterus and transplanted a limb to a subcutaneous location. Here the transplanted embryonic tissue, as well as simultaneous skin grafts from the father rabbit, showed a typical homologous tissue reaction while the pregnancy continued undisturbed. From this experiment, Woodruff concluded that in pregnancy there is no general alteration in reactivity of the mother, or lack of antigenicity of fetal tissues, but instead that the protected position of the fetus in utero guards it from an immune reaction.

For these reasons, I feel that we must ask whether the musculofascial graft technique is a valid test of immune tissue responses in the host animal. Dr. Joseph Dancis and I[4] injected minced guinea pig placental tissue, both as autologous and homologous transplants, into the subcutaneous tissues of newborn animals delivered by cesarean section. The death of both kinds of tissue occurred in about 2 weeks without vascularization of the graft, and we concluded that for this reason the homologous tissue reaction had never been invoked. In the case of musculofascial grafts, these tissues also undergo degeneration and absorption and one may question whether the tissue responses to nonvascularized dying tissue can be compared with those to a living, accepted graft.

In one sense, however, the technique described by Dr. Andresen holds great promise. In the study of fetal-maternal relationships, pregnancy itself is an extremely difficult experimental model which is conditioned by hormonal, metabolic, and

structural considerations, in addition to possible immune responses. The musculofascial graft shares with pregnancy the unique feature of failure to elicit an homologous tissue response and if this can be shown to be an indication of altered tissue reactivity of the maternal host, rather than a result of experimental technique, we will have a most useful method for exploring the entire problem.

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Dr. Edward C. Hughes, Syracuse, New York. Last year, Dr. Richard Konys, a fellow in my department, and I became interested in homographs and transplanting various types of placental tissue into the peritoneal cavity of nonpregnant rabbits with the millipore filter method. The millipore filters were 1 inch in diameter and with openings in the filter of 0.4 $\mu$. The tissue is removed from the placenta immediately after cesarean section and in some instances from the placenta as soon as it was delivered through the vagina. This tissue was then placed in a nutritive solution, taken to the laboratory where it was transferred to sterile millipore filters, and then implanted over the surface of the liver in nonpregnant rabbits. We have found that the local reaction described so well this morning took place at the edge of the transplants. We were not impressed that this filter accomplished the purpose that we thought it might. The filters were then removed each week and the tissue was studied for cellular reaction. In some instances, the placental cells did fairly well for about 4 weeks. Degeneration started in some instances as early as 2 weeks and the changes were noted particularly in the RNA and DNA factors. Dr. Konys' illustrations showed sections of the placental tissue removed at 2 weeks, 3 weeks, and 4 weeks after implantation. Examination of the tissue revealed that the syncytial cells showed early change by the fact that the RNA in the cytoplasm of the cell disappeared. The cytotrophoblasts, which were found in greater numbers in the diabetic and toxic placentas, seemed to last longer and to contain a considerable amount of glycogen, but these cells also showed a lack of RNA in the cytoplasm each week after implantation. We concluded that although we had tissue remain viable for 17 weeks, it takes more than the millipore filter to keep the reactions of the body from killing off the graft. I think Dr. Andresen has an excellent idea and his paper has been well prepared and presented.

Dr. Andresen (Closing). I wish to thank Dr. Sturgis for his kind discussion. There appears to be some question regarding the type of graft employed in these studies. Musculofascial tissues were chosen because of abundance and accessibility. Best of all, these tissues could be resected under sterile precautions and implanted in protected locales which could be critically studied by ordinary microscopic methods. Also this graft, being of simple composition, could be subjected to chemical fractionation if necessary. The response of the normal pregnant host to the homograft is, indeed, an unexplained biologic phenomenon, and many theories have been proposed to explain it. Recently, at the clinical level, we have been made aware of the fact that the human pregnant host will "tolerate" a homologous skin graft for twice as long a period as the nonpregnant host and that, if the same human subject is again skin grafted from the original donor during a subsequent pregnancy, she will exhibit an accelerated rejection reaction. There appears to be, during human pregnancy, a very reactive maternal immunologic system as related to host-homograft interactions.

Dr. Sturgis has asked whether embryonic tissues were solely used in these experiments. Placental tissues were not included. It is possible that, if placenta containing embryonic blood were so used, we might arrive at the same goal with regard to the modified homograft reaction. These experiments are already in progress. Cortisone studies have not been done. We have attempted to stay within physiologic realms and without exogenous hormonal stimulation of the host. The modifications of homologous tissue reaction, as you have seen, have been elicited by simple biologic means. It may be that the corticoids play some part in defeating the inflammatory reaction. Dr. Sturgis has also asked about the relationship of the white musculofascial graft to the white skin graft. Both of these are tissue reactions of the avascular type and appear to demonstrate a degree of severe antagonism between host and graft.

I wish to thank Dr. Douglas for his considerate comments. We have not attempted musculofascial grafting in guinea pigs or mice. In the rabbit, the fascia overlying the erector spinae is well developed. This fascial barrier appears, for an unknown reason, to be a prerequisite for eliciting classical homologous tissue reactions with skeletal muscle. It is our feeling that the musculofascial graft and its interaction with the treated host is an accurate index of previous homologous immunization. "Dead" homologous cells given to the host prior to grafting will not modify the classical homologous musculofascial graft reaction. Dr. Douglas has also asked about cortisone which I have already mentioned.

I wish to also thank Dr. Hughes. It was most interesting to learn of his experience with placental tissues in the millipore chamber.

May I again thank the Society for the opportunity of presenting experimental material.

# Endocrine studies of lactation amenorrhea

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THERE have been few studies of the hormonal alterations occurring during lactation amenorrhea. The previous reports have been concerned with the following aspects: (1) the endometrial changes encountered during lactation, (2) the interval until the first bleeding episode and the resumption of ovulation, (3) estrogen excretion, (4) the restoration of mature vaginal epithelium, and (5) studies of basal body temperature.

Clinically, we noted that certain patients during lactation complained of marked vaginal soreness and tenderness. On examination the vagina was often reddened and atrophic in appearance but no pathogens were found. Cornification studies of the vaginal epithelium revealed marked atrophic changes. These observations stimulated our interest in investigating the etiology of these alterations. The collection of 24 hour urine samples was started to determine if there were signs of ovarian failure as reflected by an elevation of pituitary follicle-stimulating hormone. Fortunately, in the first patients studied, significant levels of pituitary gonadotropins were detected. Since there had been no previous large scale studies of gonadotropin excretion during lactation amenorrhea, it was felt a detailed investigation of the hormonal findings should be undertaken.

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## Methods and materials

There were 40 lactating and 14 nonlactating patients studied. Each patient was asked to bring in a 24 hour urine sample and a vaginal smear each week until the onset of the first menstrual period. The vaginal smears were exposed to iodine vapor (Mack's stain) and judged for the type of cells present and the relative proportion of glycogen-containing cells. The cellular composition was graded from atrophic (1) to fully cornified (4) and the prevalence of glycogen-containing cells from 1-plus to 4-plus.

The urine specimens were processed for gonadotropins by adsorption on kaolin.[1] The extracts were assayed in immature female rats and the resulting ovarian weights were used as an index to relative amounts of gonadotropins. No quantitative unitage was attempted. The standard amount administered to each rat represented that aliquot of urine which contained 0.5 Gm. of creatinine. Since about 1 Gm. of creatinine per day is excreted, the test dose was approximately a 12 hour aliquot. We consider creatinine levels as an internal control which permits a more uniform comparison among specimens.

## Observations

During the first and second postpartum weeks, the extracts of urine contained chorionic gonadotropin and occasionally this was present in the third postpartum week.

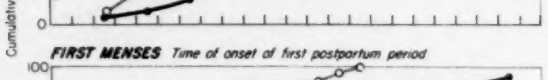


**Fig. 1. Correlation of times of vaginal glycogen response and vaginal cytologic condition to onset of first postpartum period.**

The quantities of chorionic gonadotropin were very small and were detected only because of the use of 0.5 Gm. creatinine aliquots (12 hours) while in pregnancy comparable quantities of gonadotropin are found in a 5 to 10 seconds urine aliquot. The chorionic gonadotropin found at this time represents the excretion of the hormone which has been contained in body fluids and tissues during pregnancy. This finding has not been considered pertinent to the study, and subsequent reference to gonadotropins will pertain to those of pituitary origin.

In both groups of patients by the third or fourth week after delivery the vaginal smears were generally atrophic in type (Fig. 1). Occasional red cells were found denoting the presence of lochial discharge. In nonlactating women, the vaginal epithelium was restored (a 3-plus glycogen index and precornified cells, Grade 3) by the sixth postpartum week. In lactating patients, the vaginal epithelium exhibited a 3-plus glycogen index by the sixth or eighth week but precornified cells were usually not present until the tenth week or later. Since glycogen is found in the growing cells, it is present before the beginning phases of cornification.

The first menstrual periods occurred between the sixth and ninth weeks in the nonnursing subjects whereas few nursing mothers had menstrual periods prior to the twelfth week. In the lactator, the most frequent occurrence of the first period was at 12 weeks, the average being 15 weeks because 6 mothers went beyond 22 weeks. There were 9 patients who weaned their infants during the study interval and 6 of these had their first periods in the fourth and fifth weeks after weaning.

### Endocrine studies

There were three patterns of gonadotropin excretion as noted in Table I:

Type 1. Ignoring the first 2 or 3 weeks where residual chorionic gonadotropin was detected, half of the patients had pituitary gonadotropins in the majority of specimens submitted. The levels varied from the maximal amount found in the mid-interval of a normal cycle to amounts as great as found in castrate or postmenopausal urines.

Type 2. About one third of the patients excreted gonadotropins intermittently (in less than half of the specimens submitted) during the study interval. In this group, the levels of gonadotropins were not as high.

Type 3. About one sixth of the patients displayed the third pattern in which no detectable gonadotropins were found. (For the purpose of this presentation, "no detectable gonadotropins" indicates the quantity was below the level of sensitivity of the test employed and not necessarily a total absence.)

The sequence of cellular and hormonal findings in Type 1 can best be illustrated in a series of representative cases.

The first case illustrates the findings (Fig. 2) in a patient where the period of lactation lasted 26 weeks. In the 22 weeks studied, there was insufficient ovarian activity to restore the vaginal epithelium to full cornification, despite the presence of pituitary hormones. Gonadotropins were present in all but 5 urine specimens. This represents ovaries unresponsive to gonadotropin for a period of 20 weeks. Conception occurred prior to any resumption of menstrual function.

Fig. 3 represents the sequence of events in a patient who entered the study at the time of the 6 weeks' postpartum examination. There seems to be evidence of estrogenic

stimulation of the vaginal epithelium after the tenth week. The glycogen index showed considerable fluctuation as compared to the cell types. Of particular interest is that 14 out of the 16 urine specimens had gonadotropins present, 3 of these were at castrate levels and 5 others were distinctly elevated. This represents minimal ovarian response despite the presence of excessive pituitary gonadotropins.

Fig. 4 is the graph of a patient who nursed her baby for 34 weeks prior to the first spontaneous menstrual period. From the seventh to tenth week, the patient was given 10 mg. norethynodrel daily to determine if the endometrium was responsive to exogenous hormonal stimulation during lactation. The initial biopsy was atrophic. Withdrawal bleeding occurred 4 days after the hormone was discontinued. The second biopsy revealed minimal progestational changes. The vaginal epithelium was artificially restored by the norethynodrel. After the norethynodrel-induced menstrual period, there were slight cyclic changes in the vaginal epithelium. In the 23 week interval after the hormonally induced period, there were 16 urine specimens which contained gonadotropins in quantities as great as or greater than found in a normal menstrual cycle. The ovarian response to these gonadotropins was barely adequate to restore the vaginal epithelium.

The last example of Type 1 response is shown in Fig. 5. This patient weaned her baby after 11 weeks of lactation. The vaginal epithelium had not been restored even though gonadotropins had been present in 5 out of 8 specimens tested. After weaning, the vaginal epithelium rapidly became cornified and the gonadotropin output diminished as the patient approached the first menstrual period. Whether the restoration of ovarian function was coincidental with weaning or was hastened by the cessation of lactation is not evident.

A Type 2 response is represented by Fig. 6. The first menstrual period occurred 15 weeks post partum. The first vaginal cornification response occurred at the fifth week and was associated with 3 urine specimens containing gonadotropins. The smears again became relatively atrophic. No further pituitary activity was noted until the eleventh week, at which time there was cornification of the vaginal mucosa, and the first period occurred at the fifteenth week. This intermittent production of gonadotropins and the

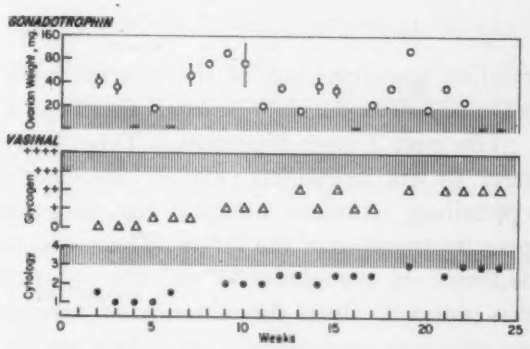


**Fig. 2.** Lactation for 26 weeks with high levels of urinary gonadotropins. Vaginal cytologic findings approached normal at the twentieth week.

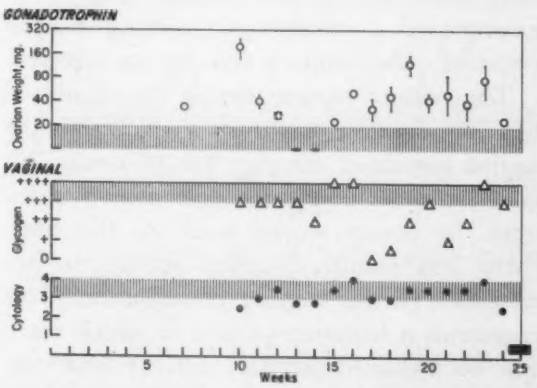


**Fig. 3.** Lactation for 25 weeks, vaginal restoration after tenth week. Note intermittent castrate levels of gonadotropins.

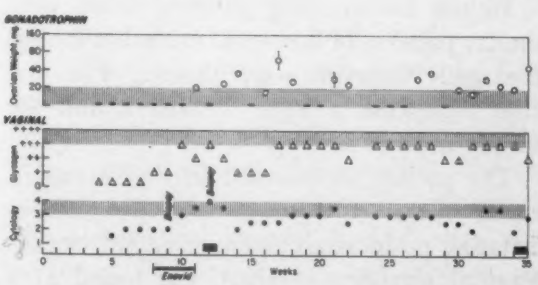


**Fig. 4.** Lactation for 34 weeks, endometrium responsive to exogenous hormonal stimulation. Vaginal restoration 17 weeks prior to first menstrual period.

**Table I.** Distribution of patients on basis of gonadotropin excretion

| | *Gonadotropins consistently present (Type 1)* | *Intermittently present (Type 2)* | *Too low to detect (Type 3)* |
|---|---|---|---|
| Lactating | 20 | 14 | 6 |
| Nonlactating | 7 | 4 | 3 |
| Time until first period (lactating) | 19.5 weeks | 13 weeks | 13 weeks |
| Time until first period (nonlactating) | 10.0 weeks | 7 weeks | 7 weeks |

relative responsiveness of the ovaries represents a different condition than the Type 1.

The next 2 cases represent a Type 3 pattern. In this individual (Fig. 7) the vaginal epithelium remained atrophic for 14 weeks prior to weaning of the infant. There was no evidence of gonadotropin excretion during this interval, thus, the absence of ovarian activity was undoubtedly secondary to deficient pituitary function. After weaning, the vagina become well-cornified within 3 weeks and gonadotropins were present in one specimen prior to the first period. This is an example of a prompt and normal ovarian response once pituitary activity was restored.

The patient represented in Fig. 8 nursed her baby for 24 weeks during which time the vagina remained atrophic for 19 weeks. No gonadotrophic hormones were demonstrated until the twenty second week. At this time, there was enough ovarian activity to accomplish partial vaginal cornification. This represents a lactating patient in which there was no apparent pituitary activity and consequently no stimulus to restore ovarian function for over 20 weeks. However, as soon as gonadotropins were present, the ovaries responded.

In the nonlactating patients, there were similar patterns of hormonal excretion though the study intervals were shorter. The first case represents a Type 1 pattern and the second a Type 3.

The patient represented in Fig. 9 experienced one of the longer intervals of nonlactating postpartum amenorrhea (14 weeks). Vaginal glycogen (3-plus) was found at 7 weeks but cornification was not evident until the tenth week. Starting at the sixth week, gonadotropins were consistently present in 9 consecutive urine specimens. This demonstrates a slow ovarian response.

The woman represented in Fig. 10 was studied until the second menstrual period. Follicle-stimulating hormone was found only in small amounts in the fourth week and the vaginal cornification was restored by the seventh week. This seems to represent an instance of very responsive ovaries. It is unfortunate that the patient omitted collecting a specimen in the twelfth week as this would have been the most probable time for demonstrating gonadotropins in the second cycle.

## Comment

During pregnancy, there are marked physiologic changes occurring in the breasts because of hormonal stimulation. The secretory activity of the breast begins about midway through pregnancy; however, copious lactation does not occur until after parturition. In pregnancy it is assumed that most of the hormones are of placental origin and

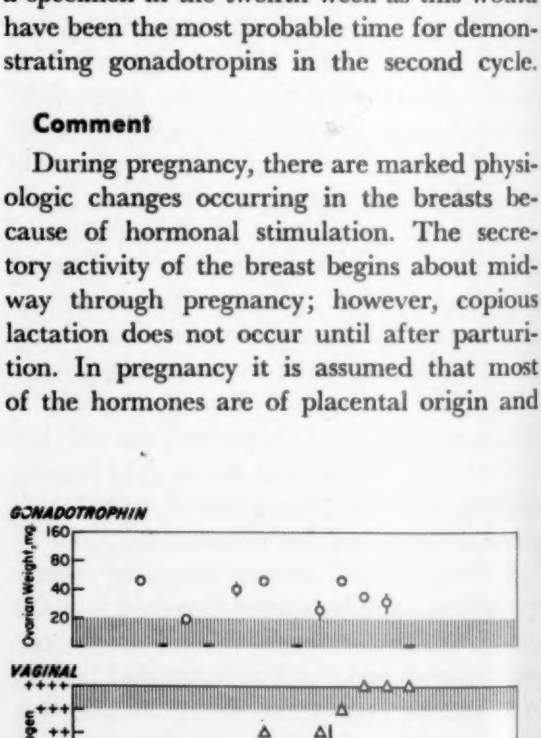


**Fig. 5.** Lactation for 11 weeks, prompt restoration of ovarian function after cessation of lactation.

the ovaries and pituitary are relatively inactive. The exact mechanism of the initiation of lactation is unknown. There are three mutually exclusive theories. Nelson[2] states that the high levels of placental estrogens suppress lactation during pregnancy by inhibiting the production of prolactin. With placental expulsion, there is a rapid decrease in the circulating estrogen, thus permitting the release of prolactin by the anterior hypophysis and the initiation of lactation. Meites and Turner[3] feel that estrogen stimulates the secretion of prolactin by the anterior lobe but in pregnancy this is prevented by the simultaneous presence of progesterone, which antagonizes the estrogen effect. After delivery, the overriding luteal influence is removed leaving the circulating estrogens free to stimulate prolactin production. Peterson[4] postulates that the secretory phase of lactation begins during pregnancy. However, the copious flow of milk following parturition is due to the ejection of the areolar contents under the influence of oxytocin secretion during labor. The milk flow thus initiated is maintained by the subsequent stimuli of sucking.

There are few studies concerning the hormonal relationships during the period of lactation amenorrhea. Peckham[5] studied the onset of menstruation in 2,885 lactating women. In 34 per cent, menses occurred 2 months after delivery. By the end of the sixth postpartum month, 70 per cent had started menstruation. The onset of the first period seems influenced by race and nutrition. Some feel that physiologic amenorrhea should not exceed 16 weeks.

Peters, Israel, and Purshottam[6] found atrophic smears only in the early months of lactation. About one half the smears taken between the fourth and twelfth postpartum months from women with lactation amenorrhea showed intermediate cornification, the rest were mature. All patients had well-cornified smears prior to the onset of menstruation. They felt smaller amounts of estrogens were needed to stimulate the growth of vaginal cells as compared to uterine epithelium.

Brown[7] found that the urinary excretions

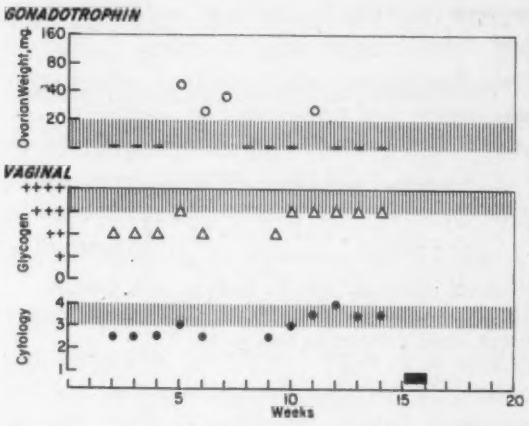


**Fig. 6.** Lactation for 15 weeks, intermittent production of gonadotropins with relative responsiveness of vaginal mucosa.

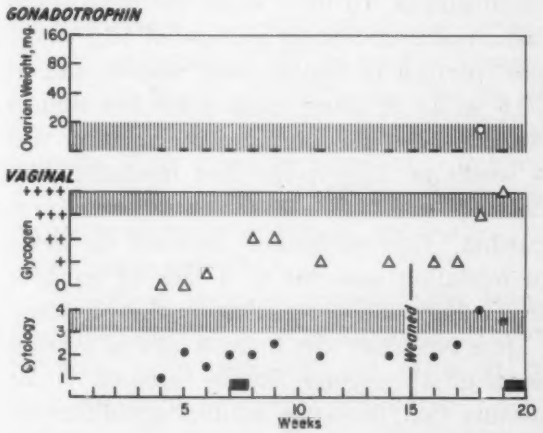


**Fig. 7.** Lactation for 15 weeks, gonadotropins absent until after weaning.

of estrogens in 4 lactating women were low and were within the same range normally found during the early part of a menstrual cycle. After cessation of lactation, the estrogen levels began to increase slowly until the regular fluctuations found during a menstrual cycle were re-established.

Udesky,[8] Topkins,[9] and Kurzrok, Lass, and Smelser[10] have studied the endometrium by serial biopsy during lactation amenorrhea. The percentage of nonsecretory changes varied. Udesky reported over 96 per cent of the endometrial biopsies studied were nonsecretory. The nonsecretory endometrium was atrophic in 15 per cent of the cases. The majority, however, showed a resting type of endometrial pattern consistent with low

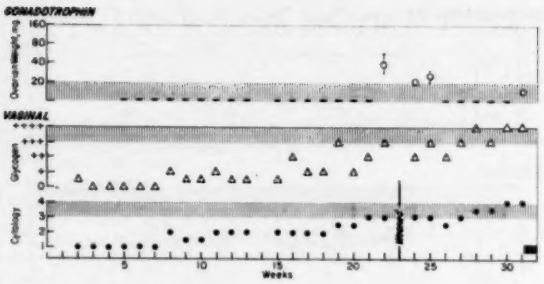


**Fig. 8. Lactation for 24 weeks, gonadotropins absent until twenty second week.**

estrogen levels. Only a very few showed full estrogenic effects.

Lyon and Stamm[11] studied the occurrence of the first ovulation by basal body temperature graphs. In their series the initial ovulations occurred at an average of 10.2 weeks post partum in nonlactating women and at 10.6 weeks in those nursing for less than 4 weeks. When the duration of lactation was 4 weeks or longer, the first ovulation was delayed until the seventeenth week post partum. They erroneously assumed the delay of ovulation was due to a lack of gonadotropic hormone production by the pituitary.

It is not clear why there is lack of ovarian or pituitary response during lactation. Some assume that prolactin inhibits gonadotropin activity, thus preventing ovarian stimulation. Others have postulated the mammary gland produces a secretion which inhibits ovarian function. They suggest there is some factor present which prevents follicular maturation.

Our most consistent finding in the vaginal smears was the basal cell and low glycogen content occurring from the fourth to sixth weeks post partum. At this time, the vagina was reddened and atrophic in appearance; several patients experienced hot flashes during this interval. These changes indicate there is an estrogen deficiency during this period because of deficient ovarian function. The duration of the low estrogen secretion varied from patient to patient and tended to be prolonged in the presence of lactation. With gonadotropic stimulation of the ovaries, the vaginal cornification was restored prior to the onset of menstruation.

The most frequent finding was the presence of gonadotropins in the majority of urine specimens. Forbes and associates[12] studied 9 normal nursing mothers and also found consistent gonadotropins in the normal range and some which were above the normal levels. The failure of the ovaries to respond to the gonadotropins suggests that the ovaries are temporarily refractory during a part of the postpartum interval. This refractory state was present in half the postpartum patients and seemed to be prolonged in the lactating patients. Unfortunately, the design of the study does not offer any evidence for cause and effect relationships in the refractory phase. The prompt onset of menstrual cycles after weaning does, however, suggest that lactation per se does delay the response of the ovaries to gonadotropin.

The absence of gonadotropins in one sixth of the patients prior to their first menstrual period suggests a deficiency of pituitary gonadotropins. The ovaries in these instances were responsive and started functioning again without prolonged stimulation. In one third of the patients, the occasional excretion of gonadotropin indicated the ovaries responded rather quickly and no significant ovarian refractoriness could be postulated. There was no difference in the average time of occurrence of the first menstrual period for Group III (no gonadotropins) and Group II (only occasional gonadotropins). Perhaps these two groups should be consolidated since urines

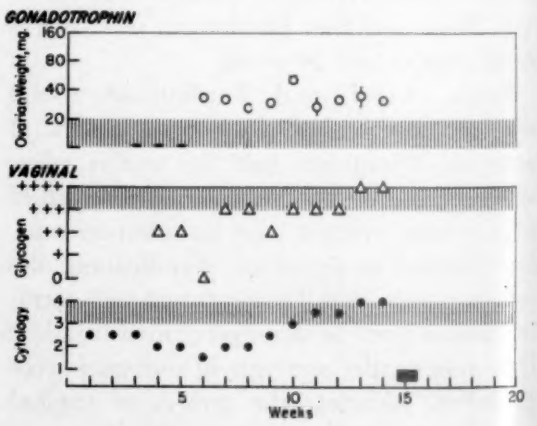


**Fig. 9. Gonadotropins present for 9 weeks prior to first menstruation.**

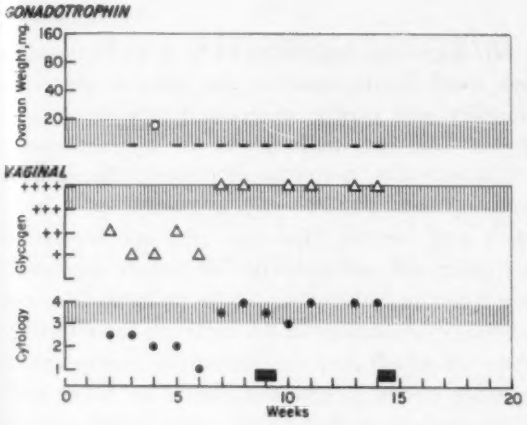


**Fig. 10.** No lactation, prompt response of vaginal mucosa. Minimal gonadotropin excretion.

were collected only once a week. The possibility exists that the random collections missed the days on which gonadotropins may have been present in Group III.

For the past 30 years we have been indoctrinated with the idea that the pituitary is the "master gland" and the ovary is a mere "slave" which always responds readily to the master's command. This concept has precluded any consideration of the possibility of a refractory state in the ovary. The refractory state is now not only a possibility but a probability. We have under observation a few young patients (nulligravidas with secondary amenorrhea and high gonadotropin levels) who are being followed to determine whether they have undergone a premature menopause or whether their ovaries are temporarily refractory. To date, one patient has resumed normal cycles.

We have noted a number of patients with secondary amenorrhea and infertility following lactation amenorrhea. None of these patients had postpartum hemorrhage or endometrial pathologic conditions. Subsequent hormonal studies have revealed absence of gonadotropins and low levels of estrogens, suggesting hypothalamic amenorrhea. These findings are similar to the hormonal changes seen in the Type 3 lactation amenorrhea.

An experimental approach to the study of the refractory ovarian state might be the prolonged administration of progestin and estrogen as in the management of endometriosis. After cessation of therapy, a study of urinary gonadotropins might reveal findings similar to those in the postpartum interval. It would be interesting but impractical to administer prolactin to determine whether it would prolong the refractory state.

## Summary

During the period of postpartum amenorrhea there seem to be three patterns of gonadotropin excretion: Type 1 is characterized by a continuous excretion of gonadotropins and a tendency toward prolonged atrophic vaginal smears indicating low estrogen production due to temporarily refractory ovaries. Type 2 is characterized by intermittent excretion of gonadotropins and a relatively early restoration of vaginal cornification indicating fairly responsive ovaries. Type 3 is characterized by the absence of gonadotropins and varied vaginal epithelial patterns. This seems to represent a delayed recovery of pituitary function. With resumption of pituitary activity, the ovaries are immediately responsive.

The three patterns occurred with equal frequency in both lactating and nonlactating patients. With lactation the duration of amenorrhea is prolonged because of either ovarian refractoriness or delayed recovery of pituitary gonadotropic activity.

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## Discussion

Dr. Howard Ulfelder, Boston, Massachusetts. It has always been known that the lactating puerperal mother resumes menses and recaptures fertility more slowly than the nonlactator. Dr. Keettel and his associates have reported their observations on 54 women, a large group, large enough indeed to let him distinguish the 3 patterns he has described to you and to conclude that both lactators and nonlactators show these differences. His data are in accord with those in other reports showing normal or slightly elevated gonadotropin excretion levels and varying degrees of estrogen deficiency in the vaginal cells. The implied discrepancy between these observations is the subject of Dr. Keettel's particular scrutiny.

The postpartum endocrine pattern has been studied in laboratory animals. Parkes and Bellerby, in 1927, concluded that lactation in mice suckling normal-sized litters somehow caused the corpora lutea of the postpartum ovulation to persist and inhibit estrus. Estrus would occur with estrogen injection, the amount necessary varying directly with the number of young sucklings. Moreover, the estrus so produced was anovulatory and, therefore, infertile. A very thorough study of the ovarian pituitary relationship in the puerperal rat by Rothchild and associates concludes with the hypothesis that a central nervous system influence inhibits luteotrophic hormone secretion and another central nervous system influence stimulates gonadotropin secretion and that sucking suppresses both central nervous system influences. He confirmed also the correspondence between the magnitude of the responses and litter size. With sucking, therefore, there is follicular quiescence. Mandl's observation in the rat raises interesting speculations. She studied the responsiveness of the rat ovary to chorionic gonadotropin and found a diminished sensitivity with adrenal insufficiency (adrenalectomy) and an increased sensitivity with thyroid deficiency (thyroidectomy). It is clear any explanation proposed for the phenomenon under study must reckon with mechanisms which control both production and release of tropic hormones by the hypophysis and by the other endocrine glands as well.

Dr. Keettel's suggestion of ovarian refractoriness must be discussed in the light of this very complex and poorly understood kaleidoscopy of events. Ovarian nonresponse of the menopause seems clear-cut, but what of puberty? Detectable gonadotropin precedes the menarche by 2 or 3 years and, indeed, Hartman long ago compared the puerperal convalescence of female reproductive function quite plausibly to puberty. Is it not necessary to consider in the lactators the effectiveness with which they are performing this function, in other words, to give some heed to "litter size" in the analysis of data? One suspects that quantitative assay of gonadotropic hormones as now possible against standard preparations will soon be carried out in patients such as these with relative ease and accuracy. Ovarian refractoriness must remain a hypothesis until a great deal more investigation has been done.

Dr. Keettel (Closing). We mentioned that there were no detectable gonadotropins in many specimens. This does not mean a total absence, but merely that they were below the level of sensitivity of the test employed.

This study was designed to obtain facts concerning pituitary activity during postpartum amenorrhea with or without lactation. Now that we recognize that there is a condition suggesting ovarian refractoriness, it is felt this condition needs further study. One type of patient which should be studied is the patient who has endometriosis and has been placed on the oral progestin compounds to prevent menstrual periods. Determination of the gonadotropins during treatment and after discontinuing the drug would be of interest.

We have been impressed by the number of patients with secondary amenorrhea after prolonged lactation. In these patients, there was no history of postpartum hemorrhage to produce pituitary changes. The hormonal findings in such patients are similar to our Type 3 response. Thus, it would appear that the so-called hypothalamic amenorrhea corresponds to that seen in the patients in our study showing no gonadotropin excretions.

# Long-term estrogen substitution and atherosclerosis

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ATHEROSCLEROSIS and its sequelae have been of increasing interest to scientific workers and clinicians. The role of cholesterol and serum lipids in the development of atheromatous changes in blood vessels is being explored widely. In the search for various factors which may influence cholesterol metabolism, gonadal function has received considerable attention. This is not surprising for cholesterol is a key substance in the production of steroid hormones.

Women during their reproductive years are relatively immune to the development of atherosclerosis and its sequelae. One of the most significant facts about coronary disease in America is that women are all but free of this serious complication prior to the menopause. The onset of the climacteric and the diminution of estrogen production coincide with the rapid and progressive increase in the incidence of atherosclerosis.

This is a preliminary report on our long-term study of several groups of women in an attempt to establish the role of endogenous and exogenous estrogens during the climacteric and following a natural or induced menopause.

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## Review of the pertinent literature

**Incidence of atherosclerosis.** Premenopausal women have a much lower incidence of coronary atherosclerosis than their age-matched male counterparts.[1] Moriyama, Woolsey, and Stamler[2] reported, in an analysis of sex differences in cardiovascular-renal mortality in the United States, that in both the white and non-white populations, the largest sex difference is in the death rates from arteriosclerotic heart disease. The mortality ratios are 1:3.2 to 1:6.5 in favor of females in the white race, and 1:1.2 to 1:1.8 in the non-white population. The rarity of coronary atherosclerosis in premenopausal women was confirmed by Gertler and associates[3] and by Glendy, Levine, and White.[4]

After the menopause, concomitant with the decreased estrogen production, as measured by urinary estrogen levels[5] and indicated by hypoestrogenic patterns of vaginal smears, there is a sharp increase in the incidence of atherosclerosis in women. By the seventh decade, the incidence of coronary atherosclerosis reaches that of men.[6, 7] A Scandinavian autopsy study showed that the occurrence of severe calcification of the abdominal aorta to be almost twice as frequent in men as in women between the ages of 50 and 60 years; between the ages of 61 and 70 years, the women had an approximately 60 per cent greater incidence of such calcification than men; and in the eighth decade the incidence of severe calcification of the abdominal aorta was twice

as great in women as in men. Roberts, Moses, and Wilkins,[1] however, deny an immunity to atherosclerosis in women in their reproductive ages at anatomic sites other than the coronary arteries.

Turning to a comparison of incidence of atherosclerosis in women with intact ovaries versus those who have undergone bilateral oophorectomy, it is again suggested that ovarian function alters the pathogenesis of this disease. Wuest, Dry, and Edwards[8] reported a study in which the hearts of 49 women who had undergone a bilateral oophorectomy were compared, with regard to the degree of sclerosis, to the hearts from 600 normal men and 600 normal women of comparable ages. On the average, the degree of sclerosis in the women who had undergone bilateral oophorectomy was greater than in the noncastrated group but less than in the male control group. All oophorectomized women in this study had menstruated regularly at the time of operation and the time interval between oophorectomy and death was greater than 2 years in all cases.

However, a similar study by Novak and Williams[9] failed to show significant differences in the incidence of arteriosclerosis for castrates and noncastrates at various decades of death, regardless of the age at operation or years intervening before death.

In a clinical study, Robinson, Higano, and Cohen[10] reported an increase in atherosclerosis in women castrated prior to 45 years of age, as compared to a similar control group who underwent hysterectomy without bilateral oophorectomy. Criteria used were clinically manifest coronary heart disease and peripheral vascular disease. Age, time elapsed since operation, obesity, and nationality were not significant factors. Hypertension, retinopathy, radiographic cardiomegaly, and diabetes were more frequent in the castrates, but could be termed only "suggestive trends." Elimination of pre-existing hypertension and diabetes mellitus did not greatly alter the increased prevalence of clinic atherosclerosis in the castrated women.

**Serum lipid patterns.** Closely related to atherosclerosis are the levels and patterns of circulating lipids. Elevated serum cholesterol, cholesterol to phospholipid (C/P) ratio and $\beta/\alpha$-lipoprotein-cholesterol ratio are often associated with coronary atherosclerosis.[11-15] It has been suggested that circulating phospholipids may act as colloidal cholesterol stabilizers, and their relative lack (as reflected by a high C/P ratio) may favor cholesterol deposition.[16] According to Boyd and Oliver,[17] "All lipids, including the sterols, are hydrophobic; hence the transparent appearance of a fasting plasma sample must be due to the presence in plasma of some constituent solubilizing the water-insoluble lipids—this is most likely a phospholipid-protein complex."

The changing patterns of incidence of atherosclerosis in women with various internal hormonal environments are closely paralleled by changes in the circulating lipids, indicating an intimate relationship between ovarian function on one hand and both lipid metabolism and atherogenesis on the other.

Even during normal physiologic hormonal fluctuations, changes in blood lipid patterns are seen. Oliver and Boyd[18] studied a group of cases of normal young women, which they followed with basal body temperature records and repeated determinations of serum cholesterol and phospholipids through several menstrual cycles. They found a striking fall in plasma total cholesterol (due entirely to a fall in plasma ester cholesterol) and a less marked fall in plasma phospholipids, with a resultant fall in the total C/P ratio occurring at midcycle (peak level of estrogen). In the follicular and luteal phases of the cycle the C/P ratios were higher because of an increase plasma total cholesterol. The lowest cholesterol levels occurred in midcycle and just prior to the menses. This study corroborated the work of Offenkrantz,[19] who in 1938 demonstrated an increase in plasma cholesterol during menstruation.

In pregnancy, a hypercholesteremia was demonstrated by several investigators

(Dieckmann and Wagner, among others) reaching a maximal level between the thirty-first and thirty-third week. This consists of an increase of approximately 50 per cent over the nonpregnancy levels and has been ascribed primarily to endocrine influences.[20] Following the menopause, a rise in plasma cholesterol occurs, reaching higher levels in the sixth decade than in the adjacent fifth and seventh decades.[21] Boyd and Oliver[21] state: "The rise in the first postmenopausal decade with a subsequent fall in the later decades has not yet been explained, and is probably related to the withdrawal of estrogens at this time. However, it is possible that any hormonal imbalance which may affect plasma cholesterol is temporary and that, subsequently, the lipids regain their former equilibrium in some way."

Other investigators have likewise reported a postmenopausal rise of serum cholesterol,[22] of cholesterol-phospholipid ratio,[11, 23] and of $\beta/\alpha$-lipoprotein-cholesterol ratio.[11]

In view of the above considerations regarding the effect of ovarian function on the circulating lipids, bilateral oophorectomy in hormonally active women would be anticipated to produce definite changes in the blood lipid levels and patterns. In a comparison of women who have had bilateral oophorectomies prior to menopause and a similar control group that underwent hysterectomy without castration, Robinson, Higano, and Cohen[10] found significantly higher serum lipid levels in the castrated group between the ages of 40 and 50 years. No difference was seen in the 51 to 60 age group. In another study,[24] the same authors found that serum lipids, including total cholesterol, phospholipids, C/P ratio, and $\beta/\alpha$-lipoprotein ratio increased with age in normal women. However, these changes were more prominent in women castrated prior to the menopause. The overlapping between the two groups was sufficient to bar any predictions on the basis of the serum lipid changes. This study was based on 39 oophorectomized patients and a comparable control group.

A similar study of 28 patients whose lipids were studied before and after castration, as well as of 24 healthy women, yielded rather inconclusive results, probably because of too short a follow-up.[25]

Oliver and Boyd[26] found that serum cholesterol levels and C/P ratios were higher in women who had undergone bilateral oophorectomy when compared to those with intact ovaries or following unilateral oophorectomy. Premature development of coronary heart disease was also present in the bilateral oophorectomy group. It was present in 25 per cent in the latter, while its incidence in normal women and those who had undergone unilateral oophorectomy was 3.5 per cent and 3.2 per cent, respectively.

**The effect of estrogen on incidence of atherosclerosis on serum lipid patterns.** In a study of autopsy records of estrogen-treated men, castrated women, and women with breast carcinoma, Rivin and Dimitroff[27] showed (1) that males treated with estrogen had less atherosclerosis than normal males; (2) that oophorectomized women had an incidence of severe atherosclerosis approaching that of the male; and (3) that the probably hyperestrogenic women (those with breast carcinoma) had less atherosclerosis than normal women. The women in this project had had an oophorectomy before the age of 50 years, and at least one year (on the average 5 years) before death. The estrogen-treated men who showed a decrease in the incidence of atherosclerosis received on the average of 75 mg. stilbestrol daily.

Eilert[28] reported on 11 women receiving estrogen therapy and compared their patterns of serum lipids to determinations made at times when estrogens had not been administered. The daily doses of hormones ranged from 0.02 to 0.10 mg. of ethinyl estradiol; 0.5 to 1.0 mg. of stilbestrol; and 10,000 to 30,000 units of estradiol. "In all cases there was a sharp reduction in the ratio of total cholesterol to lipid phosphorus during periods of estrogen administration. This change was usually effected by an elevation of serum lipid phosphorus and a fall in total cholesterol; however, in one instance the average total cholesterol level

was unchanged, and in one it actually increased. In only one patient was the lipid phosphorus during periods of estrogen administration lower than the control values, but this was accompanied by a decrease in . . . the cholesterol-lipid-phosphorus ratio." The reduction of the cholesterol-phospholipid ratio in men treated with 1 mg. of ethinyl estradiol or 15 mg. conjugated equine estrogens daily was reported by Eder.[29] Administering 0.1 to 0.2 mg. ethinyl estradiol daily to patients with idiopathic hyperlipemia and hypercholesteremia, Adlersberg[30] achieved a significant reduction of serum cholesterol level and less pronounced drop in phospholipids, indicating a reduction of the C/P ratio. Oliver and Boyd[31] showed a constant depression of hypercholesteremia and the C/P ratio in 50 estrogen-treated men over a 2½ year period; however, no significant improvement was noted over the morbidity and mortality from coronary disease of a comparable control group. Steiner, Payson, and Kendall[32] have concurred with the finding that estrogens affect the serum lipids in patients with coronary arteriosclerosis.

Effect of estrogens on serum lipids in postmenopausal women has been investigated extensively by Robinson and associates.[33] They reported in one study that a "dramatic serum lipid response was obtained in patients treated with 5 or 10 mg. of conjugated equine estrogens daily; both the cholesterol-phospholipid and the $\beta/\alpha$-lipoprotein-cholesterol ratios were reduced to levels comparable to those of normal young women. The administration of 1.25 mg. conjugated equine estrogens daily was only partially successful in altering favorably the serum lipid patterns. A dose of 2.5 mg. of conjugated equine estrogens daily lowered the C/P ratio satisfactorily, although the $\beta/\alpha$-lipoprotein-cholesterol ratios remained somewhat elevated.

In a subsequent report, Robinson, Higano, and Cohen[34] presented the following results: (1) 0.01 mg. ethinyl estradiol daily produced no effect on the serum lipids of 15 postmenopausal women. (2) Significant serum lipid changes occurred when conjugated estrogens in daily doses from 0.626 to 10.0 mg. was given. Dosages of 2.5 mg. and higher caused vaginal bleeding in most women with intact uteri. The authors, therefore, recommended conjugated estrogens in daily doses of 1.25 mg. for women with intact uteri, and 2.5 mg. in previously hysterectomized women, in order to affect favorably the serum lipid pattern in the presence of vascular disease.

Optimal changes in serum lipids reported by Marmorston and associates[35] following treatment with 0.01 mg. ethinyl estradiol have not been confirmed by these studies.

### Experimental considerations

In the experimental fields, efforts are being made to elucidate the mechanisms of hormone action on lipid metabolism, and to understand better the interrelationships among hormonal activity, blood lipid patterns, and atherosclerosis. By techniques utilizing radioactive tracer substances, the influence of hormones on lipid biosynthesis in the liver was shown by Rubin and White[36] and the effect of hormones on lipid metabolism in the aorta and on the movement of lipids between it and its nutrient fluid was demonstrated by Werthessen.[37]

Pick, Stamler, and Katz,[38] in studies on ovariectomized hens, concluded that "in association with loss of endogenous estrogen secretion, ovariectomized hens lose their resistance to cholesterol-induced coronary atherogenesis." In further studies, Pick, Stamler, and Katz[39] found similarities between experimental situations and conditions described in the foregoing clinical and pathologic studies. They concluded that in chicks, rats, and cockerels, estrogens produced a significant inhibition of cholesterol-induced coronary atherogenesis while thoracic atherogenesis was unchanged. "In some experiments thoracic atherogenesis was worse in estrogen-treated than in untreated animals, while full protection of coronary vessels was obtained." It was strongly suggested that in the chick and fat C/P ratio was closely related to the occurrence or prevention of coronary atherosclerosis. In the

rabbit, where estrogens caused no alteration of the C/P ratio, coronary atherogenesis was not inhibited. Addition of androgens to estrogens caused a lessening of the feminizing effect, but C/P ratios remained estrogenic in type. Simultaneous administration of hydrocortisone or insulin did not change the estrogen effect on the blood lipids. A euthyroid state was found necessary to allow estrogens to exert their protective function.

The present study has the following objectives:

1. To determine whether differences in serum lipid concentrations and patterns exist among the following groups of women: (a) normal premenopausal women, (b) women who underwent spontaneous menopause, (c) surgically castrated women receiving no hormone therapy, and (d) surgically castrated women receiving small doses of diethylstilbestrol.

2. To determine whether small doses of diethylstilbestrol administered over varying lengths of time influence the blood lipid changes following surgical menopause.

3. To compare the incidence of clinically evident arteriosclerotic coronary heart disease and hypertension in postmenopausal women with and without ovaries and the effect of low dosage hormone treatment on their incidence.

4. To evaluate the effect of small doses of diethylstilbestrol on the general hormonal status of castrated women, as reflected by vaginal cytology.

### Material and methods

Four groups of patients, comprising a total of 240 cases, were studied. The first group consisted of 25 women in the premenopausal period as controls. These individuals were having normal menstrual periods at the time of the study and had no climacteric symptoms. For this group mostly women in their late forties and early fifties were chosen so that their mean age would approach as closely as possible that of postmenopausal individuals. The mean age for the first group was 48.2 years.

The second group consisted of 105 women who had undergone a spontaneous menopause between 3 months and 25 years prior to the study. Their mean age was 56.1 years.

A third group consisted of 25 women who had undergone bilateral oophorectomy in conjunction with hysterectomy between 3 and 30 years prior to the study and had received no hormonal therapy since operation.

The fourth group consisted of 85 patients who likewise underwent bilateral oophorectomy at the time of hysterectomy but these women had been on estrogens since the operation. They had been taking diethylstilbestrol orally, beginning with 0.5 mg. daily about the fifth day following operation. This dose was usually reduced to 0.5 mg. every other day at the end of a year, or in younger women, after several years. The mean age for this group was 52.2 years. The time elapsed since operation ranged from 6 months to 20 years.

Tables I and II show the age distribution in each group and the distribution of pa-

**Table I.** Distribution of patients in each group by age

| *Age group* | *Premenopausal* | *Spontaneous menopause* | *Castrates* | *Castrates on hormones* |
|---|---|---|---|---|
| 38-40 | 3 | 0 | 0 | 0 |
| 40-44 | 3 | 2 | 0 | 4 |
| 45-49 | 12 | 5 | 3 | 21 |
| 50-54 | 7 | 36 | 10 | 37 |
| 55-59 | 0 | 31 | 7 | 15 |
| 60+ | 0 | 31 | 5 | 8 |
| Total | 25 | 105 | 25 | 85 |

**Table II.** Distribution of patients according to number of years past menopause

| *Years postmenopausal* | *Spontaneous menopause* | *Castrates* | *Castrates on hormones* |
|---|---|---|---|
| Less than 5 | 39 | 3 | 14 |
| 5- 9 | 34 | 8 | 50 |
| 10-14 | 21 | 6 | 18 |
| 15-19 | 9 | 5 | 2 |
| 20+ | 2 | 3 | 1 |
| Total | 105 | 25 | 85 |

tients according to time elapsed since the menopause (natural or surgical) in the last three groups.

Wherever applicable, age-corrected analyses of data were made to eliminate any possible distortion caused by uneven age distribution in the groups and were found to be closely similar to the uncorrected results.

The following studies were carried out in all patients.

**1. Total serum lipid, cholesterol, and phospholipid levels.** The total lipids were determined gravimetrically by Bloor's method[40]; cholesterol, colorimetrically by the Liebermann-Burchardt reaction[41]; and phospholipids, by measuring the lipid phosphorus according to Gomori's colorimetric technique and multiplying the values by 25 to calculate the phospholipids.[41a] No special diets were followed prior to the testing, and all blood samples were drawn after 12 hours' fasting.

**2. Fasting blood sugar determinations.** These were done in order to screen out subjects with unsuspected diabetes mellitus. Somogyi's[42] modification of the Folin-Wu method was used.

**3. Electrocardiograms.** Twelve-lead electrocardiograms were evaluated as suggested by Scarborough and associates.[43]

**4. Vaginal smears.** Vaginal smears were obtained from the majority of the women, by means of the Papanicolaou staining techniques,[44, 45] for the evaluation of the estrogenic effect on the vaginal epithelium.

**5. Physical examination.** Each patient was subjected to a complete physical examination.

Patients with histories or findings suggestive of hypothyroidism or diabetes mellitus were excluded from the study because of the possibility that these states may be associated with hypercholesterolemia and an increased incidence of coronary atherosclerosis.[46-50]

## Results

**Total plasma lipids.** The mean values for total lipids in the four groups were: 844 mg. per cent in the premenopausal controls; 996 mg. per cent in the spontaneous menopause group; 995 mg. per cent in the untreated castrates; and 1,000 mg. per cent in castrates receiving hormone therapy (Table III). The only group showing a significant difference was the premenopausal group. The women in the latter group, however, were considerably younger than those in the other three; nevertheless, comparison of values in the 45 to 49 and 50 to 54 year age brackets showed the premenopausal women to have lower total lipids than their age-matched postmenopausal counterparts. No difference was seen in the total lipids of the other groups, age bracket by age bracket.

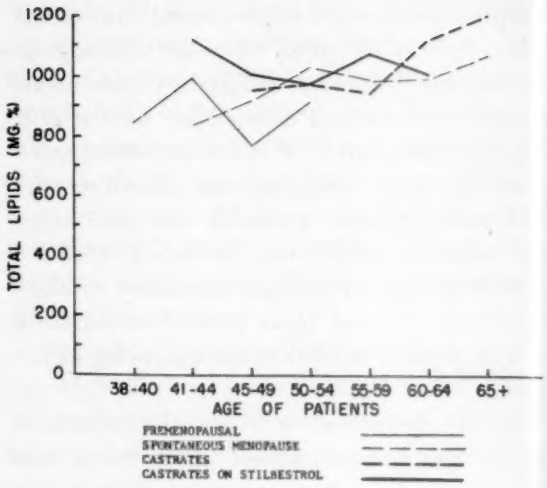


**Fig. 1.** Total lipids by age.

The total lipid levels seemed to remain stable, regardless of age, in the premenopausal group and in oophorectomized women receiving hormone therapy. The spontaneous menopause group and the untreated surgical castrates showed a tendency to a sustained rise of total lipids with age, more pronounced in the untreated castrates. In the case of premenopausal controls, at least, it seemed justifiable to postulate that the lower total lipid levels were related to the active hormonal function of the ovaries. The administration of small doses of diethylstilbestrol did not reduce the elevated total lipids in the castrated women. A noteworthy finding was that the lipid levels in hormonally untreated, surgically castrated women were no higher than those in women who under-

went a natural menopause. With reference to total serum lipids, removal of the ovaries produced changes identical to those caused by a natural menopause. Fig. 1 is a graphic representation of the total serum lipids by age groups in each category of subjects.

The above results were also analyzed in terms of hyperlipemia. Table IV summarizes the percentage of women in each group and in each age bracket whose total plasma lipids exceeded 1,000 mg. per cent. In the premenopausal group the incidence of hyperlipemia was 16 per cent; the other three groups were again closely similar, the incidence of hyperlipemia averaging 43.1 per cent. Within the four groups, trends were observed which were similar to those described above in connection with the actual total lipid values.

When the total plasma lipid concentrations of the four groups were compared according to time elapsed since menopause, some lowering of the values below those of the spontaneous menopause group and untreated castrates was observed in the hormone treated castrates when 10 years or more have elapsed since the menopause. Table V and Fig. 2 show these data.

**Serum cholesterol.** The mean values for serum cholesterol in the 4 groups were: 221 mg. per cent in the premenopausal controls; 272 mg. per cent in the spontaneous menopause group; 271 mg. per cent in the untreated castrates; and 267 mg. per cent in castrates receiving hormone therapy (Table III). As with total plasma lipids, the only group showing a significant difference in cholesterol levels was the premenopausal group. Within each group, no significant relationship between cholesterol levels and age was noted, but a suggestion of a slow and small rise with age was present. In order to eliminate the age factor in the youngest members of the premenopausal group, comparison was made of cholesterol levels in matching age brackets; namely, in the 45 to 49 and 50 to 54 year age groups. Again, the levels remained low in the premenopausal group, even when compared to age-matched subjects in the other three categories. The spontaneous menopause group, the untreated castrates, and the castrates on hormone therapy all had similar cholesterol levels, both as total groups and age-matched subdivisions. Effect of ovarian hormones on the serum cholesterol levels could be postulated only in the premenopausal group. The administration of small doses of diethylstilbestrol did not prevent the rise of cholesterol levels after oophorectomy. However, it was again noted that the removal of ovaries produced changes not at all different from those caused by natural menopause. Fig. 3 summarizes the above results.

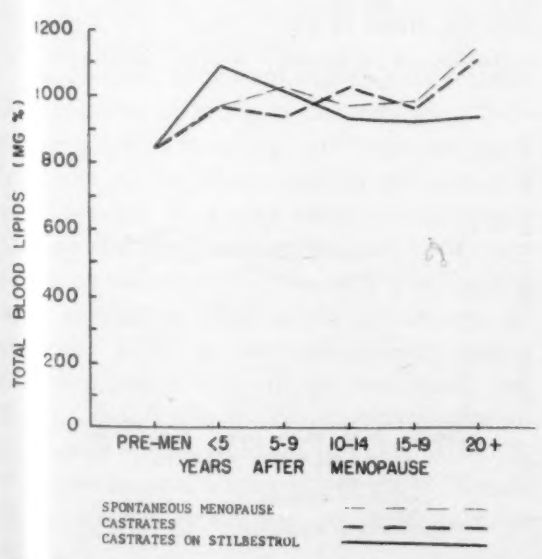


Fig. 2. Total lipids by years after menopause.

When these data were analyzed according to the incidence of hypercholesterolemia, the premenopausal group again stood alone with only 8.0 per cent of the subjects having a serum cholesterol level over 300 mg. per cent. All hypercholesterolemic patients in this group were in the 50 to 54 years age bracket. The incidence of hypercholesterolemia in the spontaneous menopause group and in the untreated castrates was almost identical, 32.4 per cent and 32.0 per cent, respectively. The incidence of hypercholesterolemia of only 23.5 per cent in the hormonally treated castrates may have been a reflection of a cholesterol-lowering effect of diethylstilbestrol. The foregoing data is summarized in Table VI.

**Table III.** Plasma lipids, serum cholesterol, phospholipids, and C/P ratios (mean totals—mg. per cent)

| | *Total lipids* | *Cholesterol* | *Phospholipids* | *C/P ratios* |
|---|---|---|---|---|
| Premenopausal | 844 | 221 | 219 | 1.034 |
| Spontaneous menopause | 996 | 272 | 269 | 1.034 |
| Castrates | 995 | 271 | 264 | 1.055 |
| Castrates on hormones | 1000 | 267 | 284 | 0.986 |

**Table IV.** Serum lipids by age (per cent of cases above 1,000 mg. per cent)

| *Years* | *Premenopausal* | *Spontaneous menopause* | *Castrates* | *Castrates on hormones* |
|---|---|---|---|---|
| 38-40 | 33.3% | – | – | – |
| 40-44 | 33.3% | 0 % | – | 75.0% |
| 45-49 | 0 % | 40.0% | 50.0% | 47.4% |
| 50-54 | 28.6% | 41.7% | 33.3% | 41.2% |
| 55-59 | – | 37.1% | 14.3% | 46.7% |
| 60-64 | – | 46.7% | 100.0% | 37.5% |
| 65+ | – | 71.4% | 100.0% | – |
| Total | 16.0% | 42.7% | 41.7% | 45.0% |

Fig. 4 shows a comparison of serum cholesterol levels in the three postmenopausal groups according to time elapsed since menopause. A small, steady increase of serum cholesterol with time elapsed since menopause was seen in the women after natural menopause and in the untreated castrates. In the treated group, the cholesterol levels seemed to remain more stable and somewhat below those in the other two groups when 10 or more years have elapsed since menopause. Table VII summarizes the incidence of hypercholesterolemia according to the number of years elapsed since menopause.

**Phospholipids.** The mean values for plasma phospholipids were: 219 mg. per cent in the premenopausal controls, 269 mg. per cent in the spontaneous menopause group, 264 mg. per cent in the untreated castrates, and 284 mg. per cent in castrates receiving hormone therapy (Table III). Premenopausal women had considerably lower plasma phospholipids than the other 3 groups, while the hormonally treated castrates showed a definite tendency toward higher levels. Fig. 5 presents a comparison of plasma phospholipid levels in age-matched subdivisions of the 4 groups. By and large, the differences seen in the total mean values were also present when individual age brackets were compared. Within the spontaneous menopause groups, as well as in hormonally treated castrates, the phospholipids tended to rise slowly with age until 60 years, and above that age they had slight tendency to level off or even fall below the levels of the sixth decade. On the other hand, the rise in phospholipids with age continued rather strikingly in the untreated castrates, reaching, after 60 years of age, levels higher than those seen in any other group at any age.

The percentage of patients in each group with plasma phospholipids in excess of 300 mg. per cent was calculated. Table VIII summarizes the results. Only 4.2 per cent of the premenopausal controls fell into this category. Of the spontaneous menopause group, 28.2 per cent had phospholipid values over 300 mg. per cent; of the untreated castrates, 24.0 per cent; and of castrates on stilbestrol, 38.3 per cent. In the spontaneous menopause group no significant change with age was demonstrated in terms of incidence of phospholipid levels over 300 mg. per cent; in the untreated castrates, a significant increase of incidence was seen, in keeping with the trend in Fig. 5, while in the treated castrates, a relatively stable incidence of

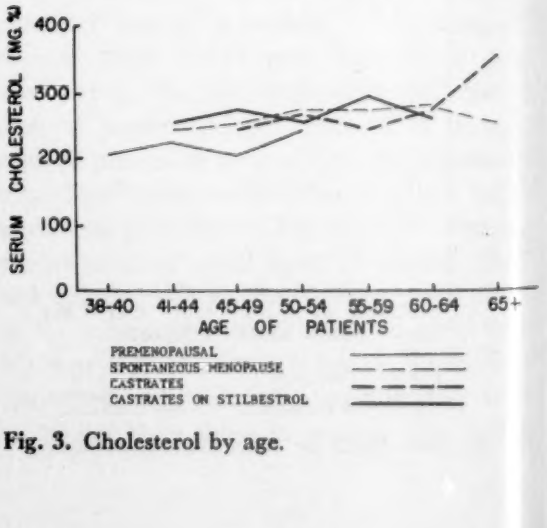


**Fig. 3.** Cholesterol by age.

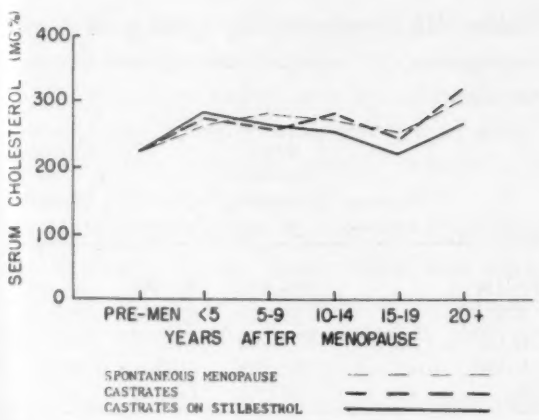


Fig. 4. Cholesterol by years after menopause.

values over 300 mg. per cent occurred well into the sixth decade, with a decreased incidence thereafter, again in keeping with the pattern seen in the representation of the mean values in Fig. 5.

As presented in Fig. 6, correlating plasma phospholipid levels with the number of years elapsed since menopause showed no differences in the three postmenopausal groups. A review of Table IX, however, showed that in the hormonally treated group the incidence of phospholipid concentrations in excess of 300 mg. per cent decreased sharply and consistently as the time interval elapsed since menopause became greater. In the spontaneous menopause group and in the untreated castrates no significant changes in the incidence of elevated phospholipids occurred with the increase in time interval elapsed since menopause.

**Cholesterol to phospholipid (C/P) ratio.** The relation of serum cholesterol to plasma phospholipid concentrations was expressed in terms of the cholesterol to phospholipid (C/P) ratio. This was calculated for each patient and the four groups were then compared to one another. The mean C/P ratios were as follows: 1.034 in the premenopausal controls, 1.034 in the spontaneous menopausal group, 1.055 in the untreated castrates, and 0.986 in the stilbestrol-treated oophorectomized subjects (Table III). A tendency toward a lowering of the C/P ratio in the treated group was suggested by these data.

In the premenopausal group, exactly 50 per cent had C/P ratios above 1.000 and 50 per cent had ratios below 1.000; no significant difference from this distribution was noted in the spontaneous menopause group and in untreated oophorectomized women. In the stilbestrol-treated group, a shift toward lower C/P ratios is suggested. Almost 56 per cent of the subjects in this group had C/P ratios below 1.000 and approximately 44 per cent were above unity.

No significant change with age in the C/P ratio was demonstrated within each group, with the exception of a fairly consistent rise with age in the castrates receiving hormone therapy (Fig. 7).

Correlating the C/P ratios with the number of years elapsed since menopause, the values in the natural menopause group were relatively low and remained stable through

**Table V.** Serum lipids by years past menopause (per cent of cases above 1,000 mg. per cent)

| *Years* | *Premenopausal* | *Spontaneous menopause* | *Castrates* | *Castrates on hormone treatment* |
|---|---|---|---|---|
| | 16.0% | – | – | – |
| Under 5 | – | 31.6% | 33.3% | 69.2% |
| 5- 9 | – | 51.5% | 42.8% | 42.6% |
| 10-14 | – | 38.1% | 33.3% | 41.2% |
| 15-19 | – | 55.6% | 20.0% | 0 % |
| 20+ | – | 100.0% | 100.0% | 0 % |
| Total | 16.0% | 42.7% | 41.7% | 45.0% |

**Table VI.** Cholesterol by age (per cent of cases above 300 mg. per cent)

| *Years* | *Premenopausal* | *Spontaneous menopause* | *Castrates without hormones* | *Castrates on hormone treatment* |
|---|---|---|---|---|
| 38-40 | 0 | – | – | 0 |
| 40-44 | 0 | 0 | – | 0 |
| 45-49 | 0 | 20.0% | 33.3% | 28.6% |
| 50-54 | 28.6% | 38.9% | 30.0% | 16.2% |
| 55-59 | – | 25.8% | 0 | 46.7% |
| 60-64 | – | 37.5% | 50.0% | 12.5% |
| 65+ | – | 28.6% | 100.0% | – |
| Total | 8.0% | 32.4% | 32.0% | 23.5% |

the years. As seen in Fig. 8, during the first 10 years following oophorectomy (without subsequent hormone treatment) the C/P ratios were comparable to those of the spontaneous menopause group. After 10 years, however, there was a rather pronounced and sustained elevation of the ratio. The stilbestrol-treated castrates maintained ratios comparable to those of the natural menopause group.

**Incidence of electrocardiographic abnormalities and hypertension.** The incidence of abnormal electrocardiograms is shown in Table X. The occurrence of abnormal electrocardiograms was rather uniform in all groups except for the hormone-treated castrates and approached approximately 10 per cent of the cases. In the hormone-treated oophorectomized subjects, however, the total incidence of abnormal electrocardiograms was less than 5 per cent. The most marked difference was observed in women below the age of 55 years and in those who were 10 years or less postmenopausal. In the age groups above 55 years and in women who had passed their menopause by 10 years or more, the incidence of electrocardiographic abnormalities was fairly uniform in all four groups.

No significant difference in the incidence of hypertension was found in the four groups of patients as a whole, but a conspicuously low incidence was observed in hormone-treated castrates 10 years or more postmenopausal (Table XI). No significant differences were noted with regard to occurrence of hypertension, when the patients were divided into age groups, one below 55, the other 55 and over.

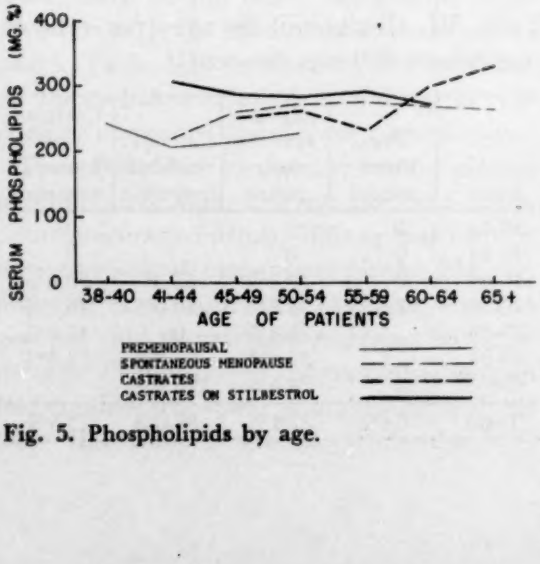


Fig. 5. Phospholipids by age.

**Table VII.** Cholesterol by years past menopause (per cent of cases above 300 mg. per cent)

| *Years* | *Premenopausal* | *Spontaneous menopause* | *Castrates* | *Castrates on hormone treatment* |
|---|---|---|---|---|
| | 8.0% | – | – | – |
| Under 5 | – | 30.8% | 33.3% | 43.6% |
| 5-9 | – | 35.3% | 25.0% | 22.0% |
| 10-14 | – | 33.3% | 50.0% | 16.7% |
| 15-19 | – | 22.2% | 0% | 0% |
| 20+ | – | 50.0% | 66.6% | 0% |
| Total | 8.0% | 32.4% | 32.0% | 23.5% |

**Table VIII.** Phospholipids by age (per cent of cases above 300 mg. per cent)

| *Years* | *Premenopausal* | *Spontaneous menopause* | *Castrates* | *Castrates on hormone treatment* |
|---|---|---|---|---|
| 38-40 | 0 | – | – | – |
| 40-44 | 0 | 0 | – | 50.0% |
| 45-49 | 0 | 40.0% | 0 | 45.0% |
| 50-54 | 14.3% | 31.4% | 10.0% | 40.0% |
| 55-59 | – | 33.3% | 14.3% | 33.3% |
| 60-64 | – | 16.7% | 50.0% | 14.3% |
| 65+ | – | 28.6% | 100.0% | – |
| Total | 4.2% | 28.2% | 24.0% | 38.3% |

## Comment

The results of our preliminary study on the long-term administration of estrogens as substitution therapy in the postmenopausal period as an aid in the retardation of the development of atherosclerosis present significant considerations.

Overwhelming clinical and experimental evidence supports the relationship of the levels and patterns of circulating lipids to the pathogenesis of atherosclerosis. The role of cholesterol, and especially of its levels in relation to serum phospholipids, is gaining wide acceptance. Therefore, we feel that both the alterations in serum lipid patterns

seen with changes in the individual's hormone status (be they brought about endogenously or exogenously) and the concomitant changes in incidence and severity of clinically evident arteriosclerotic disease are parts of the same process.

Our results follow the trends that one would expect on the basis of previous work of other investigators. Following the administration of stilbestrol we found a definite tendency toward a lowering of serum cholesterol and toward an elevation of phospholipids, while the total circulating lipids remained relatively constant. The C/P ratios were thus lowered by stilbestrol administration and appeared to be a fairly sensitive index of the estrogenic effect.

The small degree of changes in the serum lipids, as well as the lack of conclusive differences in the occurrence of clinical arteriosclerosis and hypertension, may, in part, at least be attributed to three factors. In the first place, the period of follow-up most likely was not long enough to allow a complete exhibition of all trends. This study will be continued and we hope that it will provide additional evidence to support the patterns presented in this report.

In the second place, although our results are statistically significant, our samples are obviously small, especially in the nontreated surgical castrate groups. Again, with a larger number of patients, our results may become more significant. It will be most interesting to compare large numbers of untreated surgical castrates with women who have undergone a spontaneous menopause. Our preliminary findings did not confirm the differences in these two groups reported by other workers. Last, the dosage of estrogen used was much smaller than the dosage reported by others. Although some studies described favorable effects on the blood lipids in the administration of comparably small doses, most of the dramatic responses reported were obtained with doses many times the amounts we administered.

The dosage employed, 0.5 mg. stilbestrol every other day for continued long-term administration, was selected primarily in an effort to obtain adequate substitution with no undesirable side effects. Obviously, in women with uteri, breakthrough (escape) bleeding occurs frequently with larger doses

Table IX. Phospholipids by years past menopause (per cent of cases above 300 mg. per cent)

| *Years* | *Premenopausal* | *Spontaneous menopause* | *Castrates* | *Castrates on hormone treatment* |
|---|---|---|---|---|
| | 4.2% | 0 | – | – |
| Under 5 | – | 26.3% | 33.3% | 64.3% |
| 5-9 | – | 36.4% | 12.5% | 40.4% |
| 10-14 | – | 14.3% | 33.3% | 17.6% |
| 15-19 | – | 33.3% | 0 | 0 |
| 20+ | – | 50.0% | 66.6% | 0 |
| Total | 4.2% | 28.2% | 24.0% | 38.3% |

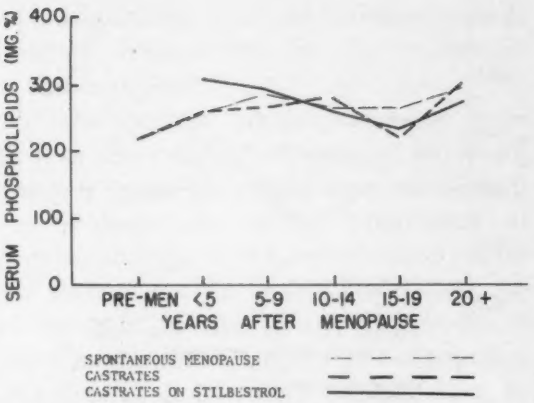


Fig. 6. Phospholipids by years after menopause.

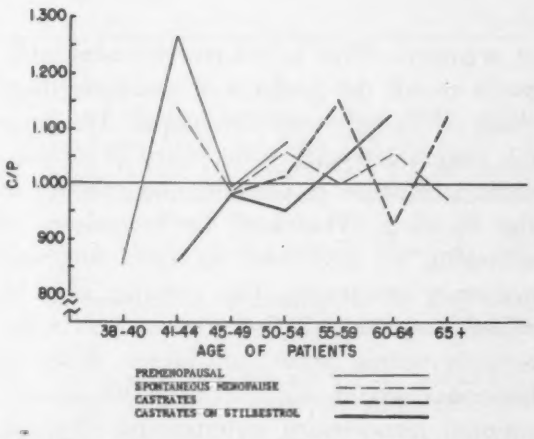


Fig. 7. Cholesterol-phospholipid (C/P) ratio by age.

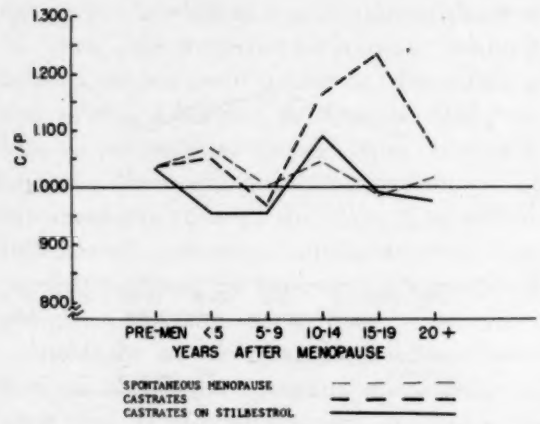


**Fig. 8.** Cholesterol-phospholipid (C/P) ratio by years after menopause.

of estrogens. This is extremely undesirable, for it clouds the problem of the early diagnoses of endometrial carcinoma. We have felt that a curettage is necessary in such instances in order to establish the etiology of the bleeding. Therefore, by low doses of estrogens, we have tried to avoid such unnecessary curettages. The question may be raised, however, whether the benefit (in the antiatherogenic sense) of larger doses of hormones would not far outweigh an occasional unnecessary curettement. Perhaps our conservative approach to the administration of estrogens to postmenopausal women with intact uteri, and the quantity of the hormone administered, should be revised.

Finally, the kind of estrogen used may be of importance, although all available evidence points to the conclusion that favorable serum lipid patterns and alterations in incidence of atherosclerosis can be obtained with a variety of natural and synthetic compounds. In the treatment of men, compounds with minimal feminizing influences are being sought to replace the current estrogens in order to minimize the undesirable side effects when used in the male to combat atherosclerotic manifestations.

At our institution, we have been using diethylstilbestrol since its introduction to clinical medicine.[51, 52] As the first synthetic compound available for oral administration, we find it unsurpassed in convenience of administration, effectiveness, relative freedom from undesirable side effects and toxicity, and its consistently favorable action on the vaginal mucosa.

## Summary

1. Premenopausal women have significantly lower total serum lipid, serum cholesterol, and phospholipid levels than their age-matched counterparts in the other three groups of patients under study.

2. The levels of total lipids, cholesterol, and phospholipids in untreated surgical castrates were practically identical to those in women who had undergone a spontaneous menopause.

3. Statistically, significant trends toward lower cholesterol and higher phospholipid levels were observed in the oophorectomized women on stilbestrol therapy. Total lipids remained relatively unchanged.

4. The cholesterol-phospholipid (C/P) ratio was constant in the premenopausal, spontaneous menopausal, and oophorectomized (untreated) groups. It was lowered in the castrates receiving stilbestrol.

5. The incidence of abnormal electrocardiograms was lower in the hormonally treated castrates than in untreated women,

**Table X.** Abnormal electrocardiograms by years past menopause (per cent of cases)

| *Years* | *Spontaneous menopause* | *Castrates* | *Castrates on hormone treatment* |
|---|---|---|---|
| Less than 10 | 9.6% | 9.1% | 1.6% |
| 10 or more | 18.8% | 7.1% | 14.3% |
| Total | 12.4% | 8.0% | 4.8% |

**Table XI.** Hypertension (150/100 +) by years past menopause (per cent of cases)

| *Years* | *Spontaneous menopause* | *Castrates* | *Castrates on hormone treatment* |
|---|---|---|---|
| Less than 10 | 12.3% | 9.1% | 14.1% |
| 10 or more | 18.8% | 14.3% | 4.8% |
| Total | 14.3% | 12.0% | 11.8% |

both naturally postmenopausal and oophorectomized.

6. When 10 or more years had elapsed since the menopause or operation, stilbestrol-treated women had a lower incidence of hypertension than untreated patients.

7. The incidence of abnormal electrocardiograms and hypertension was closely similar in untreated patients, regardless of whether the menopause was spontaneous or surgically induced.

8. The incidence of atrophic vaginal smears was remarkably similar in the untreated postmenopausal subjects, both non-oophorectomized and oophorectomized. It occurred infrequently in the stilbestrol-treated castrates.

9. The sporadic administration of estrogens for the relief of menopausal symptoms is widely used. Their long-term administration is important in the retardation of atrophic changes in the reproductive organs and throughout the body. Their most important role, however, may be their use to retard atherosclerosis and its serious sequelae. This area of prophylactic therapy must be explored more widely in the hope that women may retain good health in their advancing years.

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## Discussion

Dr. Clyde L. Randall, Buffalo, New York. I have appreciated the privilege of reading the entire paper to be published by Dr. Davis and an opportunity to consider the data he has presented. We should remember he has emphasized today that these observations should be considered as a preliminary report.

It is interesting to note, however, even 10 or more years after a spontaneous menopause, Davis found that only 18 per cent of the patients had developed hypertension. Last year 34 per cent of the supposedly normal women regarded by Novak and Williams as controls were reported to have developed a marked degree of arteriosclerosis in their 50's, and 74 per cent had developed such a marked arteriosclerosis by their 60's. Either a number of the noncastrates Davis has accepted as controls have atherosclerotic changes without hypertension or the noncastrated women regarded as controls in the Hopkins study included an unusually large number of women with arteriosclerosis.

Additional observations are needed with careful interpretation of the data being published. One source of confusion can be eliminated if we remember that ovarian removal and the withdrawal of estrogens cannot be regarded as one and the same thing. Even 10 to 15 years after bilateral oophorectomy the tissues of nearly half of all castrates continue to show evidences of estrogenic effects, presumably from extraovarian sources of estrogen.

Although the data reported by Davis has not been offered as conclusive evidence, a number of his observations support previously reported studies. Since variations in the quantity of estrogens received as replacement therapy seem associated with significant differences in cholesterol-phospholipid metabolism, there is evident need for additional effort to determine the dosage of estrogens adequate to protect the castrate or the postmenopausal woman from the progress of atherosclerotic disease. Robinson has recommended a dosage equal to 2.5 mg. of conjugated equine estrogens daily if hysterectomy has rid the patient of the risk of endometrial bleeding. If the uterus has *not* been removed and estrogens are to be given in an effort to retard the extent and severity of vascular disease, Robinson has recommended not less than 1.25 mg. of conjugated equine estrogens or equivalent dosage daily.

I suspect many of you are inclined to conclude that at the time of indicated hysterectomy we might well remove both ovaries prophylactically and prescribe estrogens to prevent the undesirable degrees of atrophic and atherosclerotic changes which, without such replacement therapy, at least 50 per cent of castrates can be expected to develop.

Our own experience, however, with women advised to continue on such replacement therapy does not support some of the claims made for the effectiveness of the "pills." Some patients *will* enthusiastically and with evident benefit continue such a prescription indefinitely; others apparently resent being advised to do so, and like the too fat girl who defiantly continues to eat too much, some castrates keep discontinuing the

very therapy that would prevent their difficulties. We find it difficult to believe that a majority of patients will dutifully and faithfully continue to do what is best for them over a period of 10 to 20 years as evidenced by the fact that 20.5 per cent of the stilbestrol-treated castrates in Davis' own series were reported to show atrophic smears. Was this a matter of dosage or did some patients fail to take their pills?

I have been disappointed not to hear consideration of one observation I thought might have been included in Novak's data last year and one I would like to have found in the observations reported today by Davis. Why not take one group of women, regardless of the etiology of the menopause they experienced or the fact that they may have been given a prescription for estrogens, but a group all of whom *show atrophic smears* and compare them with similar numbers of women of equal ages whose smears do *not show evidences* of estrogen deficiency? If maintenance of an estrogen level sufficient to avoid atrophic vaginal smears will reduce the frequency and severity of atherosclerosis, this fact should be apparent in such a comparison.

Let us not forget that after 45 years of age 83 per cent of cervical malignancies and over 90 per cent of the carcinomas destined to begin in the endometrium or ovary have still to make their appearance.

We should also remember that 8 of the only 9 women among each 1,000 who eventually develop ovarian malignancy do so after their fiftieth year, a fact we should consider in view of the probability that vascular accidents and the degenerations due to arteriosclerotic disease account for more deaths than all types of malignancies combined.

Unfortunately, preserving the ovary may only delay atherosclerotic changes for a decade or two. Nevertheless, premature castration is likely to remove even the estrogen delaying protection against atherosclerotic disease which approximately half of all women lose if they do lose their ovaries. If prophylactic ovarian removal is good for the patient at 35 years of age, we believe that it is likely to be almost as good for her at 50, after which age at least the serious consequences of early castration need no longer be considered. Such studies as this one, however, appear to be the one way we can eventually realize really intelligent attitudes in regard to the questions of prophylactic ovarian removal, and the advisability of estrogen replacement after their menopause for that proportion of women whose tissues evidence a lack of estrogen soon after cessation of menstruation.

Dr. Russell R. de Alvarez, Seattle, Washington. I am interested in some of the comments which Dr. Davis has made today relative to the influence of estrogen on the production of atherogenesis in elderly individuals. If we look at this problem in another way, one would wish to consider the influence of estrogen on the production of atherogenic plaques and thus the influence of estrogen on the production of elevation of the systemic blood pressure. In our approach toward this problem, we carried out a serial study of lipids in normal pregnancy in order to make comparisons with pre-eclampsia-eclampsia, a situation where an elevation of blood pressure occurs. In normal pregnancy there does occur a simultaneous increase in circulating an excreted estrogen along with an increase in the total lipids and all the lipid fractions. This rise ordinarily does not make its appearance until the sixteenth or the twentieth week of pregnancy and, once it does, the elevation is sustained throughout the pregnancy. One then would wish to determine if there is a relationship among the production of estrogen, the lipogenesis of pregnancy, and the production of elevated blood pressure in toxemia of pregnancy. It has been reported by some that there is an increase in all the lipid fractions and the total lipids in toxemia of pregnancy, but the mistake is made in comparing the lipid levels in patients with severe toxemias of pregnancy with the values in a normal nonpregnant patient. This is not correct because when comparisons are made simultaneously for a given period of pregnancy in a patient with toxemia or normal pregnancy there is no statistically significant difference in the lipid values between the normal pregnant patient and the patient with toxemia of pregnancy at the same duration of pregnancy. The only change, an increase, is in the cholesterol-phospholipid ratio.

I should like to ask Dr. Davis about the other fractions. Has complete fractionation of all the lipids been carried out? What is the influence of lipoproteins? Is anything known about the fractionation of lipid which makes up this lipoprotein? Does he mean that there is a sustained production of estrogen augmented exogenously which produces an increase in lipid in some of these patients who develop evidence of atherogenesis or are the two mutually independent?

Dr. Davis (Closing). I wish to thank Drs. Randall and de Alvarez for discussing our paper. This is a preliminary report of a study which we hope can be continued for a sufficiently long period to determine the role of estrogens in the retardation of atherogenesis in women. Our data indicate that estrogen substitution following the production of endogenous estrogens may be a very important factor in slowing the development of the sequelae of atherosclerosis. If this proves to be true, we must consider the administration of exogenous estrogens to women who no longer have ovarian function for the rest of their lives.

Dr. Randall has raised the question of ovarian function during pelvic surgical procedures. You are aware of our thinking in this regard. No one would belabor the question of safeguarding ovarian function in the woman prior to the menopausal period. However, when the removal of the uterus is necessary in an individual in the forties, that period of waning ovarian function, and certainly in the postmenopausal period, it has been our belief that ovarian conservation is unrewarding. There is no doubt that ovaries continue to produce decreasing amounts of estrogens for several years or longer. However, the amount soon becomes insufficient to retard "aging." The remaining adnexa following operation in middle life has a very limited period of usefulness. Estrogen production decreases rapidly. It may become cystic and painful. It may develop neoplastic changes and, although malignancy is not a frequent complication, when it does occur, it is often a fatal disease.

The introduction of orally effective synthetic and natural estrogens some 20 years ago stimulated us to re-evaluate our approach to gonadal conservation at pelvic operation. We demonstrated that it was possible to administer exogenous estrogens safely and physiologically. The removal of adnexa at hysterectomy allows immediate institution of substitution therapy. It suppresses menopausal symptoms thereby favoring a good recovery, promotes healing, and maintains the normalcy of the vagina and external genitals. With few exceptions it can be maintained indefinitely. If additional studies support the role of estrogens in the retardation of atherogenesis, there is little doubt that long-term administration of the hormone to most women will become standard practice.

In answer to Dr. de Alvarez's question, we did not determine lipoproteins in our laboratory studies. Dr. Jones and his group in cardiology, who have been interested in the problem of atherosclerosis, felt that the quantitative determinations of these lipid fractions would not contribute much to our comprehensive study.

# The effects of chemotherapy and irradiation on ovarian and cervical cancer cells in tissue culture

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IN THE past decade the methods of tissue culture have progressed to a remarkable degree. The progression from the relatively crude, unreliable estimates of explant tissue culture[1] to the quantitative methods of monolayer cell culture[2] has allowed precise determinations of cell population in culture vehicles. More recently, plating techniques[3] involving the development of macroscopic clones from single cells has permitted the assessment of reproductive capacity of explanted cells under measured conditions of stress.

The increasing ease and reliability of methods which result in the significant growth of neoplastic tissues in culture has led to the justifiable optimism that eventually these methods can be used as effective clinical tools. However, it is a long jump from a cell strain in the test tube to cancer in the patient. To date, one of the significant drawbacks of quantitative cell culture lies in the necessity of employing established cell strains in order to obtain numerically precise results. Cell strains established over a period of years in the laboratory will reproduce with predictable accuracy, but, unfortunately, reactive variations between the various cell strains are exceedingly difficult to demonstrate. Work in our laboratory[4] and that of Ludovici and associates[5] has indicated that under certain conditions differences between the various cell strains can be demonstrated. Also, increased reliability in obtaining significant growth increments during the initial period following introduction of tissue into culture has been demonstrated. It is during this critical early phase of the establishment of a cell strain that the cells appear to retain the characteristics of their original tissue of origin.

With the intent that it will eventually be possible to work with relatively freshly cultured tissue, we have persisted in the development of methodology and have made determinations with established cell strains. It seems probable that within the foreseeable future it will be possible to employ the methods described below on cells freshly prepared from surgical material.

In a previous publication[6] we evaluated the effects of certain alkylating agents on ovarian and cervical cancer cells cultivated in vitro. No clinical application could be made from these observations since little variation between the reactive behavior of the several cell strains was demonstrated. It was possible, however, to assess the relative cytotoxic effectiveness of the different

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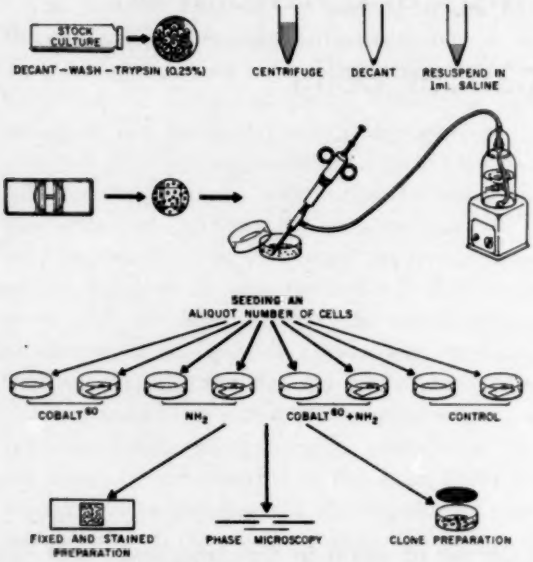


**Fig. 1.** Schematic representation of the procedure for preparation of clone culture plates.

chemotherapeutic agents in clinical concentration under tissue culture conditions. In this presentation further observations on the action of alkylating agents on cells in culture are described. In addition, observations concerning the effects of $Co^{60}$ irradiation alone and in combination with alkylating agents under these conditions are reported. It is hoped that in the near future these methods will be useful in assessing the sensitivity of freshly explanted cells.

## Material and methods

**Maintenance of stock cultures.** The cell strains utilized in these experiments have been maintained in our laboratory over a period of years. They include a strain of human amnion cells (amnion F.L.) established in the Virus Laboratory at the University of California, the cervical cancer cell strain (HeLa) established by Dr. George Gey at Johns Hopkins University, and an ovarian cancer cell strain (3-59) established in our laboratory. The cells have been maintained on the glass substrate of 200 ml. square, screwcap (milk dilution) bottles with a growth medium consisting of 90 per cent Eagle's basal media and 10 per cent calf serum. The culture bottles were kept in stationary horizontal positions so as to allow 10 ml. of culture media to cover the cell layer and they were incubated at 36.5° C. Refeeding with fresh culture media was scheduled according to the number of cells per bottle, condition of the cells, and the pH of the culture. Between $1 \times 10^5$ cells (initial inoculum) and $6 \times 10^6$ cells (confluent growth at harvest) were maintained in the stock bottles. Cells were harvested for experimental use when a confluent monolayer of healthy-appearing cells was attained. Following the initial seeding this amount of growth usually required 7 to 12 days.

**Preparation of replicate cell count and clone count cultures.** An appropriate stock culture was selected and the growth media decanted. After washing with two 5 ml. changes of saline A solution,* 5 ml. of 0.25 per cent trypsin (1:300) in saline A was added to the stock bottle and, by means of a rubber policeman, cells were gently freed from the glass. Through gentle pipetting with a 5 ml. serologic pipette, cell clumps were dispersed to form a uniform cell suspension. After centrifuging at 1,000 r.p.m. for 1 minute, the trypsin solution was decanted, the cells were resuspended in 1 ml. of saline A and a hemocytometer cell count was made. The cells were carefully diluted with saline A to provide inocula of suitable cell density.

Cell population experiments were carried out by seeding a given number of cells in control and experimental vehicles and adding sufficient growth medium to make up the desired volume (5 ml. for 60 mm. Petri dishes and 10 ml. for 200 ml. milk dilution bottles). In these experiments $10^3$, $10^5$, or $10^6$ cells were seeded according to the requirements of the experiment.

Preparations designed to evaluate the effect of exposure times of alkylating agents were set up by seeding control and experimental culture vehicles with counted cell inocula and adding growth media contain-

*Physiologic saline without calcium, magnesium, and phosphate buffer (after Puck[3]).

ing varying concentrations (0.008 to 0.12 mM.) of thioTEPA. Untreated growth media was added to the controls. The reason for using these concentrations of thioTEPA has been previously reported. Following incubation for the prescribed period of exposure (1 to 24 hours), the media of experimental and control cultures was decanted, the adherent cell layer was washed with saline A, new growth media without the alkylating agent was added, and the preparations were replaced in the incubator. At the end of the prescribed period of growth, the cultures were again washed with saline A, trypsinized, and counted in the manner described above. Comparing the cell counts of the experimental cultures with those of the controls allowed an assessment of the effect of exposure time on cell population.

In evaluating the effect of ionizing irradiation on cell population, experimental and control vehicles were quantitatively inoculated with cells, the prescribed growth medium added, and 3 to 4 hours allowed for the cells to settle on the glass substrate. Experimental cultures were exposed to 100 to 500 r of ionizing irradiation utilizing a $Co^{60}$ source at a distance 75 cm. over a 15 sq. cm. area, delivered at a rate of 50.9 to 43.3 r per minute. No supplemental oxygen was supplied during or following the irradiation procedure. Incubation was maintained at 36.5° C. in an atmosphere containing 5 per cent $Co_2$. An evaluation of the effect of irradiation was made by cell counts of both experimental and control cultures at the end of each 24 hour period of incubation following irradiation.

Clone count preparations required that a much smaller number of cells (200 to 600) be seeded in each culture so that greater care was employed to insure accurate dilution and delivery of aliquots. Also, in order to insure a respectable plating efficiency (clones formed/cells seeded), it was necessary to allow only minimal exposure to trypsin solution and to utilize only the gentlest methods in effecting cell transfers from stock bottle to culture vehicles. In general, the clone plating method of Puck[3] was followed. A carefully measured aliquot of the enumerated cell suspension was transformed to a magnetic stirrer flask containing a measured volume (e.g., 250 c.c.) of growth media. Replicate volumes of the cell/media suspension were delivered into 60 mm. Petri dishes by means of a Coleman automatic pipette (Fig. 1). In experiments wherein irradiation effect on clone formation was to be assessed, the cultures were allowed to settle to a monolayer for 3 to 4 hours and the experimental cultures were exposed to $Co^{60}$ in the manner described above. In determining the effects of alkylating agents on clone formation, the cytotoxic agent was added to the growth media in sufficient quantity to make up the desired concentration (usually 0.012 mM.) of thioTEPA. Following the prescribed period of incubation (6 or more days) for each group of cultures, all Petri dish preparations were fixed in Bouin's solution for 20 minutes, washed in tap water, and stained with Giemsa solution for 3 minutes. A 47 mm. grid prepared from a Millipore filter placed on the Petri dish facilitated counting of the macroscopic clones. Fig. 2 illustrates the appearance of such a plate of clones with the grid in place. In each case the effect of the experimental condition was evaluated by comparing the number of clones in the experimental cultures with that of the control cultures. Because of the possible variance in plating efficiencies, the control cultures had to be prepared from the same cell pool, treated in precisely the same manner, and cultured exactly concurrently as the experimental cultures.

## Results

**Effect of time of exposure to thioTEPA on cell strains.** In a previous publication[6] we reported the effects on the proliferative capacity of established cell strains when they were grown in culture media containing concentrations of thioTEPA varying from 0.008 to 0.12 mM. In these experiments the cells were exposed to the alkylating agent for the entire duration of the experiment.

Table I indicates that when the culture medium contained thioTEPA only during the first 24 hours of incubation, the effect on the cell population measured at 144 hours was not significantly different from that when the cells were exposed to the cytotoxic agent over the entire course (144 hours) of incubation. Fig. 3 illustrates graphically that with a concentration of 0.062 mM. of thioTEPA in the culture media, the effect on cell population measured at 24 hour intervals is almost identical whether cells have been incubated with thioTEPA for 24 hours or for the entire period of culture. Similar observations confirmed this finding for thioTEPA concentrations of 0.008, 0.015, and 0.031 mM. These data would indicate that the cytotoxic effect of thioTEPA on proliferating cells under the conditions described takes place during the first 24 hours of exposure.

In an effort to further define the exposure period of thioTEPA necessary to exert a measurable effect on the proliferative capacity of cell strains in vitro, the alkylating agent was removed from the culture media of replicate cultures after exposure periods varying from 1 to 24 hours. This effect was accomplished by removing the culture media after the prescribed period of exposure and substituting media containing no thioTEPA for the remaining period of incubation. Control cultures whose media contained no alkylating agent were manipulated through the same media changes as the experimental cultures.

**Fig. 2. Clone plate preparation with overlying 47 mm. grid to aid in counting. (Actual size.)**

Fig. 4 indicates that little effect on the cell population of ovarian cancer cells (3-59) measured at 144 hours was noted until the exposure time to the alkylating agent had reached 14 hours. Although this figure represents the minimal effective exposure time for 0.015 mM. of thioTEPA, the figures are entirely comparable for other concentrations within the "clinical" range. As the exposure time was increased from 13 to 22 hours, there was a progressive increase in the cytotoxic effect. With the latter exposure time, the maximal depressive effect on cell proliferation had occurred and no additional effect on cell population was noted by maintaining the thioTEPA in the media for longer periods.

**Development of tolerance to thioTEPA by cell strains in tissue culture.** Fig. 5 illustrates the progressive tolerance developed by ovarian cancer cells (3-59) to repeated sublethal concentrations of alkylating agent. As expected from previous experiments, the first exposure to 0.008 mM. of thioTEPA resulted in a 29.2 per cent depression in cell population measured at 144 hours of incubation and as compared with untreated control cultures. Following recovery from the effects of thioTEPA and culturing back to confluent growth in normal culture media, cells were again exposed to the same sublethal concentration of thioTEPA for another 144 hour period. On the second exposure the depressive effect on cell proliferation was reduced to 23.4 per cent. By the fifth successive exposure to 0.008 mM. of thioTEPA the depressive effect was only 10.2 per cent as compared with untreated control cultures. It is of interest that a similar development of tolerance by the same cell strain to sublethal doses of ionizing irradiation could not be demonstrated (see below).

**The effect of varying doses of $Co^{60}$ irradiation on ovarian cancer cells in vitro.** The progressive decrease in the ability of single cells to form clones (as shown in Fig. 2) following exposure to increasing doses of $Co^{60}$ irradiation is indicated in Table II. The cells were irradiated 3 to 4 hours following seeding in order to allow time for cells to settle into a uniform monolayer prior to irradiation. This effect is shown graphically in Fig. 6 and indicates the straight line (semilogarithmic) relationship between the dose of irradiation and the capacity of cells to clone under these conditions. Table III documents the reproducibility of results in replicate experiments for each dose of ionizing irradiation. It is noted that under these conditions of irradiation and cell culture the $ID_{50}$ dose of irradiation is very close to 200 r.

In an experiment utilizing 125 separate culture preparations of ovarian cancer cells (3-59) exposed to 200 r of irradiation, the cloning capacity of the cells (evaluated after 6 days of culture) was reduced by an average of 51.1 per cent when compared with 25 unirradiated control cultures. The standard deviation from the mean of this group of values was ± 2.75 per cent. In a similar study utilizing 125 cultures, adding 0.012 mM. of thioTEPA to the media, the cloning capacity was reduced to 50.4 per cent with a standard deviation from the mean of ± 2.9 per cent. When 125 cultures were exposed to both of these $ID_{50}$ effects (200 r $Co^{60}$ and 0.012 mM. thioTEPA), the cloning capacity of the seeded cells was reduced to 36.1 per cent of the controls with a standard deviation of ± 2.6 per cent. Fig. 7 indicates the appearance of clone cultures subjected to $ID_{50}$ exposure of thioTEPA, $Co^{60}$ irradiation, and a combination of the two.

It is of interest that the effect of irradiation and thioTEPA on cells in culture is such that the ability of the cells to undergo cell division is not always immediately affected. In some instances a cell survived the period of the experiment but, instead of forming a macroscopic clone, it underwent only 2 or 3 cell divisions following irradi-

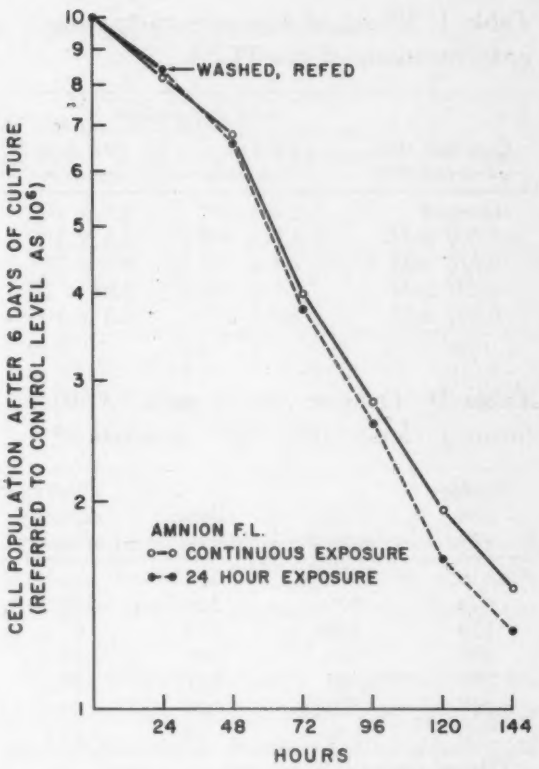


**Fig. 3.** Graph indicating that exposure to 0.062 mM. thioTEPA during first 24 hours of culture depresses cell population just as effectively as continuous exposure over the entire period of culture.

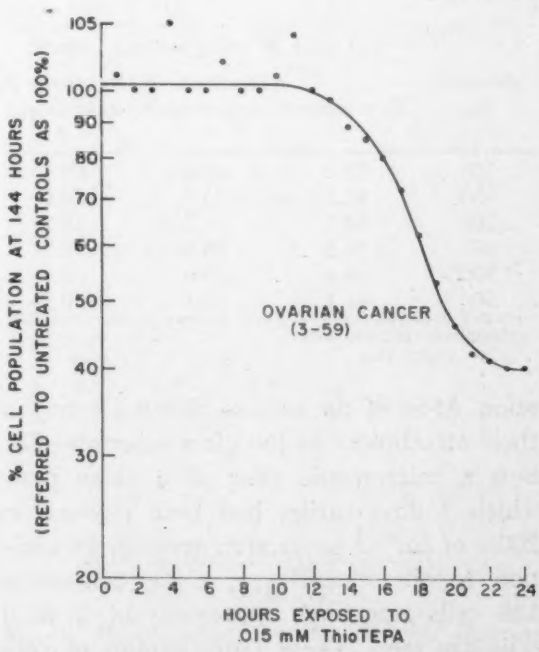


**Fig. 4.** Differential exposure graph indicating the effect on cell population of hourly exposure increments to 0.015 mM. thioTEPA in ovarian cancer cell (3-59) cultures.

**Table I.** Effect of exposure to varying concentrations of thioTEPA

| *Concentration of thioTEPA* | *Cell population* | |
|---|---|---|
| | *24 hours exposure* | *144 hours exposure* |
| Control | 2.6 x $10^6$ | 2.7 x $10^6$ |
| 0.008 mM. | 2.1 x $10^6$ | 2.0 x $10^6$ |
| 0.016 mM. | 7.5 x $10^5$ | 7.5 x $10^5$ |
| 0.031 mM. | 5.1 x $10^5$ | 5.05 x $10^5$ |
| 0.062 mM. | 1.4 x $10^5$ | 1.3 x $10^5$ |

**Table II.** Ovarian cancer cells (3-59) forming clones after $Co^{60}$ irradiation*

| *Irradiation (r)* | *Cells seeded* | *Clones formed* | *Cloning capacity of cells†* |
|---|---|---|---|
| 0 | 500 | 411 | — |
| 100 | 500 | 324 | 83.2 |
| 150 | 500 | 277 | 67.5 |
| 200 | 500 | 198 | 48.2 |
| 250 | 500 | 157 | 38.3 |
| 300 | 500 | 124 | 30.4 |
| 500 | 500 | 43 | 11.1 |

*Plating efficiency, 82 per cent.
†Control, 100 per cent.

**Table III.** Effect of $Co^{60}$ irradiation on clone formation by ovarian cancer cells (3-59)

| *Irradiation (r)* | *Per cent of cells forming clones** | | |
|---|---|---|---|
| | *Experiment 1* | *Replicate 1, experiment 2* | *Replicate 2, experiment 3* |
| 100 | 83.2 | 81.8 | 82.4 |
| 150 | 67.5 | 65.0 | 66.9 |
| 200 | 48.2 | 51.7 | 48.9 |
| 250 | 38.3 | 35.9 | 36.3 |
| 300 | 30.4 | 30.0 | 28.4 |
| 500 | 11.1 | 10.4 | 9.5 |

*Control, 100 per cent.

ation. Most of the cells so affected soon lost their attachment to the glass substrate. Fig. 8 is a microscopic view of a clone plate which 7 days earlier had been exposed to 200 r of $Co^{60}$ 3 hours after seeding. In addition to the macroscopic clone containing 128 cells numerous aggregates of 2 to 4 cells are seen. These latter groups of cells were not grossly visible and would not have been counted in the usual clone count. Such findings suggest that cells so affected by irradiation or cytotoxic compounds sometimes continue to divide several times following injury without necessarily retaining their capacity to form clones.

As indicated in Fig. 9, when ovarian cancer cells (3-59) were repeatedly exposed to sublethal ($ID_{50}$) doses of $Co^{60}$ irradiation, no significant development of tolerance could be discerned. On each occasion after the exposed cells were nursed back to stock bottle confluency, the cloning capacity of the irradiated cells as compared with that of the unirradiated controls was still depressed by 50 per cent.

**Qualitative effects of $Co^{60}$ irradiation and thioTEPA on ovarian and cervical cancer cells in vitro.** In order to illustrate the qualitative cytologic effects of ionizing irradiation on cells in culture, large inocula of cells ($1 \times 10^5$) were seeded into Petri dishes containing coverslips and subjected to $Co^{60}$ irradiation, thioTEPA in the media, and combinations of the two injurious agents. The coverslips with cells attached were prepared for cytologic examination after 6 days of incubation. As noted in Table IV, cell counts of the cultures were made at 24 hour intervals during the first 144 hours of culture. In general, the ovarian cancer (3-59) and HeLa cell strain of cervical cancer each reacted in a numerically similar manner. Fig. 10 graphically illustrates the effect on ovarian cancer cells (3-59) and indicates that the depressive effect of ionizing irradiation on cell population takes place somewhat sooner than that of the alkylating agent. However, at the end of 144 hours of

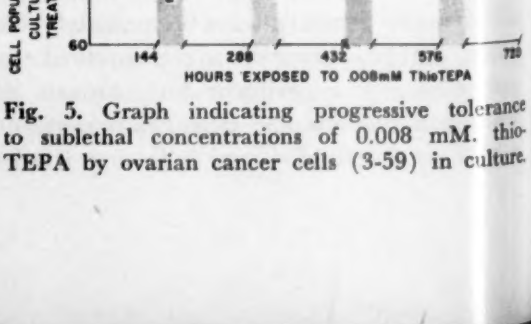


**Fig. 5.** Graph indicating progressive tolerance to sublethal concentrations of 0.008 mM. thioTEPA by ovarian cancer cells (3-59) in culture.

Table IV. Effects of thioTEPA, $Co^{60}$, and both on ovarian and cervical cancer cells in vitro

| Cell type | Percentage of cell population* | | | | | |
|---|---|---|---|---|---|---|
| | 24 hours | 48 hours | 72 hours | 92 hours | 120 hours | 144 hours |
| *Ovarian (3-59)* | | | | | | |
| 0.012 mM. thioTEPA | 98.0 | 87.6 | 68.2 | 57.6 | 54.0 | 51.2 |
| 200 r $Co^{60}$ | 76.9 | 72.2 | 64.1 | 52.5 | 48.6 | 49.0 |
| thioTEPA plus $Co^{60}$ | 69.3 | 63.0 | 48.8 | 40.0 | 37.7 | 35.9 |
| *HeLa (Gey)* | | | | | | |
| 0.012 mM. thioTEPA | 96.6 | 84.1 | 66.4 | 56.8 | 55.5 | 48.7 |
| 200 r $Co^{60}$ | 70.8 | 70.3 | 65.3 | 58.2 | 53.3 | 49.7 |
| thioTEPA plus $Co^{60}$ | 67.5 | 66.8 | 55.8 | 48.3 | 40.4 | 38.6 |

*Control, 100 per cent.

incubation the $ID_{50}$ effects of the two injurious agents approached the same figure.

Fig. 11 illustrates the microscopic appearance of ovarian cancer cells (3-59) affected by the $ID_{50}$ doses of $Co^{60}$ irradiation and thioTEPA. The appearance of the coverslip preparations in general reflected the expected decrease in cell number as compared with the controls. Whereas the primary effect noted following exposure to the alkylating agent was cytoplasmic shrinking and nuclear swelling, the effect of ionizing irradiation was largely on the nucleus with an increase in the numbers of multinuclear cells and aberrant nuclear forms. When the cells were subjected to both agents, nuclear aberrations became extreme and the development of long spinous cytoplasmic extensions from cell to cell became a prominent feature of the preparation.

## Comment

In considering the results of these experiments, it is immediately apparent that they are applicable to the clinical situation only in a general sense. Although the chemotherapeutic agents and the ionizing irradiation were qualitatively the same as those used clinically and even though they were employed in concentrations roughly comparable to those delivered to the body tissues in practice, there is little comparability between the gaseous and fluid environment of the cell culture and the internal milieu of the intact organism; nor is there a great deal of similarity between the cells selected through innumerable laboratory transfers and those freshly explanted from body tissues.

Cells selected through a process of repeated transfer in the laboratory are highly specialized. As a result of this process of selection, those cells capable of surviving

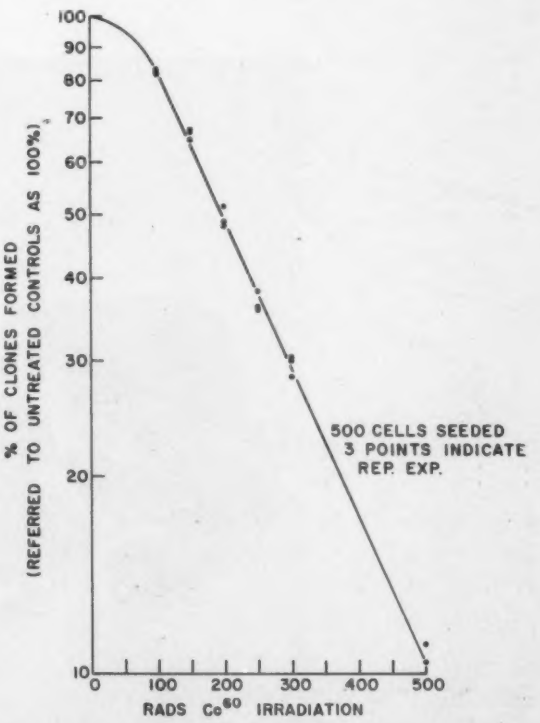


Fig. 6. Graph indicating the progress decrease in ability of single ovarian cancer cells (3-59) to form clones following exposure to increasing doses of $Co^{60}$ irradiation. Note straight line relationship under semilogarithmic growth conditions.

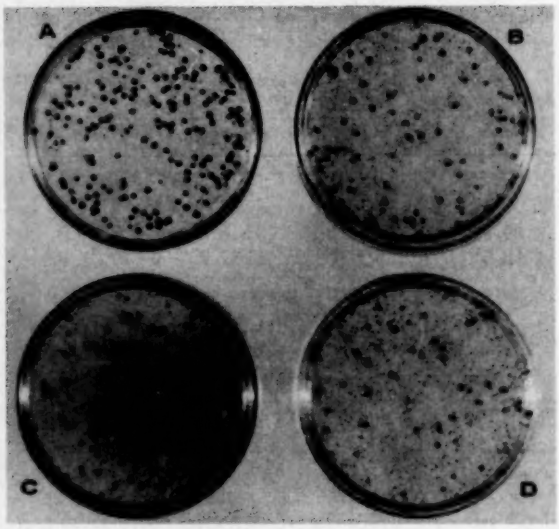


**Fig. 7.** Photograph (reduced one half) indicating the appearance of clone cultures exposed to $Co^{60}$ irradiation, thioTEPA, and a combination of the two. *A*, Control; *B*, $ID_{50}$ (0.012 mM.) thioTEPA; *C*, $ID_{50}$ (200 r) $Co^{60}$; and *D*, combination of 200 r $Co^{60}$ and 0.012 mM. thioTEPA.

and reproducing under the conditions of laboratory culture are the ones which eventually make up the cell strains and which characteristically reproduce with accurate predictability. The longer the strain has been exposed to this process of differential selection, the greater are the chances that it will behave admirably in culture and the more likely it is that the cells will differ materially from those originally derived from their tissue of origin. These differences are well illustrated in the different chromosome number found in cells of selected laboratory cell strains when compared to their chromosome number at the time they were taken directly from the body tissues. Indeed, it has been shown that, as these cells specialize in culture, they even develop differing immune responses from those that they exhibited shortly after explantation from their tissue of origin.[7]

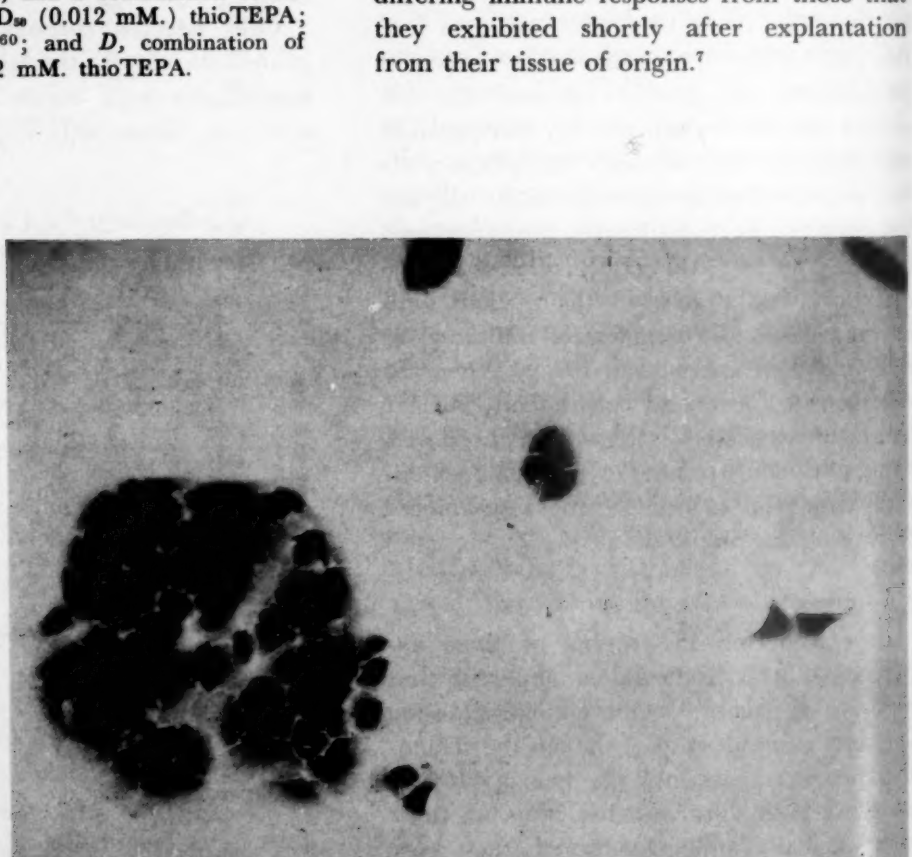

**Fig. 8.** Microscopic view of clone plate which 6 days earlier was exposed to 200 r of $Co^{60}$ 3 hours after seeding. In addition to the macroscopic clone containing about 128 cells, note the numerous aggregates of 2 to 4 cells. They probably represent abortive divisions following irradiation. (×75.)

However, it is important that the methodology be maintained, repeatedly applied, and developed further. The techniques of maintaining and developing cell cultures are continually being improved. The present techniques of cell plating with clone formation would seem to reflect accurately the ultimate ability of a single cell to reproduce its kind indefinitely. Utilizing these techniques, Elkind and his group[8, 9] have done a monumental work in determining the nature of x-ray damage to cells in tissue culture. Such techniques represent a significant advance beyond cell population appraisals and constitute a tremendous step past the crude methods of classical explant tissue culture. At the present time in order to employ the clone culture techniques successfully, the methods must be extremely refined, special fluid and gaseous media are essential, and gentle cell manipulation is mandatory.

In order for tissue culture determinations to be even remotely applicable to a given clinical situation, it is important that the information derived from it be obtained while decisions concerning the choice of therapy are being made or at least while the therapy is being carried out. It seems essential that the information be obtained with cells freshly explanted from the tissue taken at surgical excision while the cells still maintain the biologic characteristics of their in vivo form. Unfortunately, it is impossible at present to follow these techniques with freshly obtained cells and still obtain quantitatively precise results.

Fortunately, the methods of cell culture are such that fairly significant qualitative appraisals of cell growth can be obtained from freshly obtained tissue when they are minced and plated into Petri dish cultures. The reliability of this method is not yet such as to permit clinical dependance, but it is improving steadily. At the present time we are making assessments of the sensitivity of neoplastic tumors to chemotherapeutic agents as discs of the various cytotoxic agents are placed on a culture plate of cells freshly explanted from surgically excised tissues. As the methods are refined and as it becomes possible to grow cells with a numerically reproducible proliferative capacity shortly after their initial explanta-

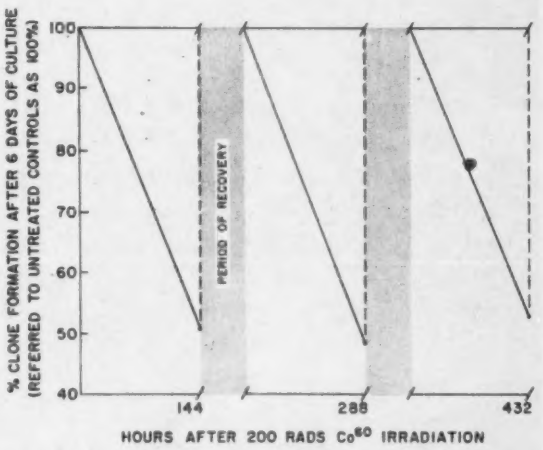


**Fig. 9.** Graph showing the effect of three successive sublethal ($ID_{50}$) doses of $Co^{60}$ irradiation. No indication of the development of tolerance is seen.

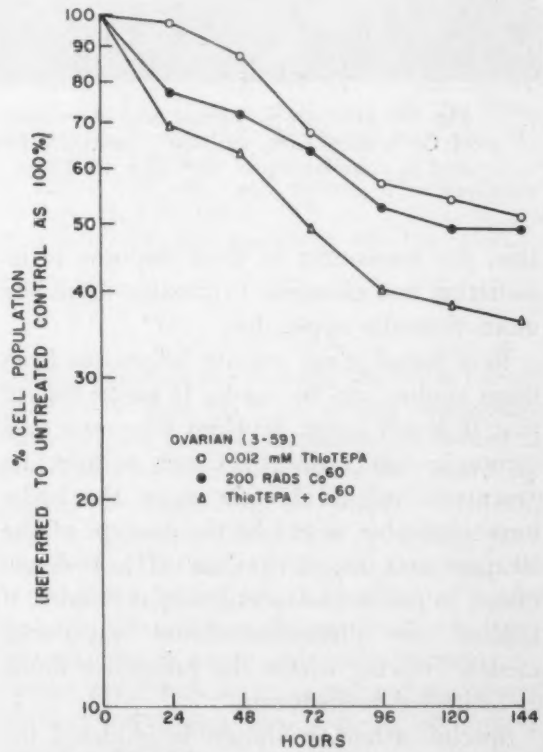


**Fig. 10.** Graphic illustration of the depressive effect on cell population of thioTEPA and $Co^{60}$ irradiation measured at 24 hour intervals. Note that the effect of irradiation appears sooner than that of a comparable dose of thioTEPA.

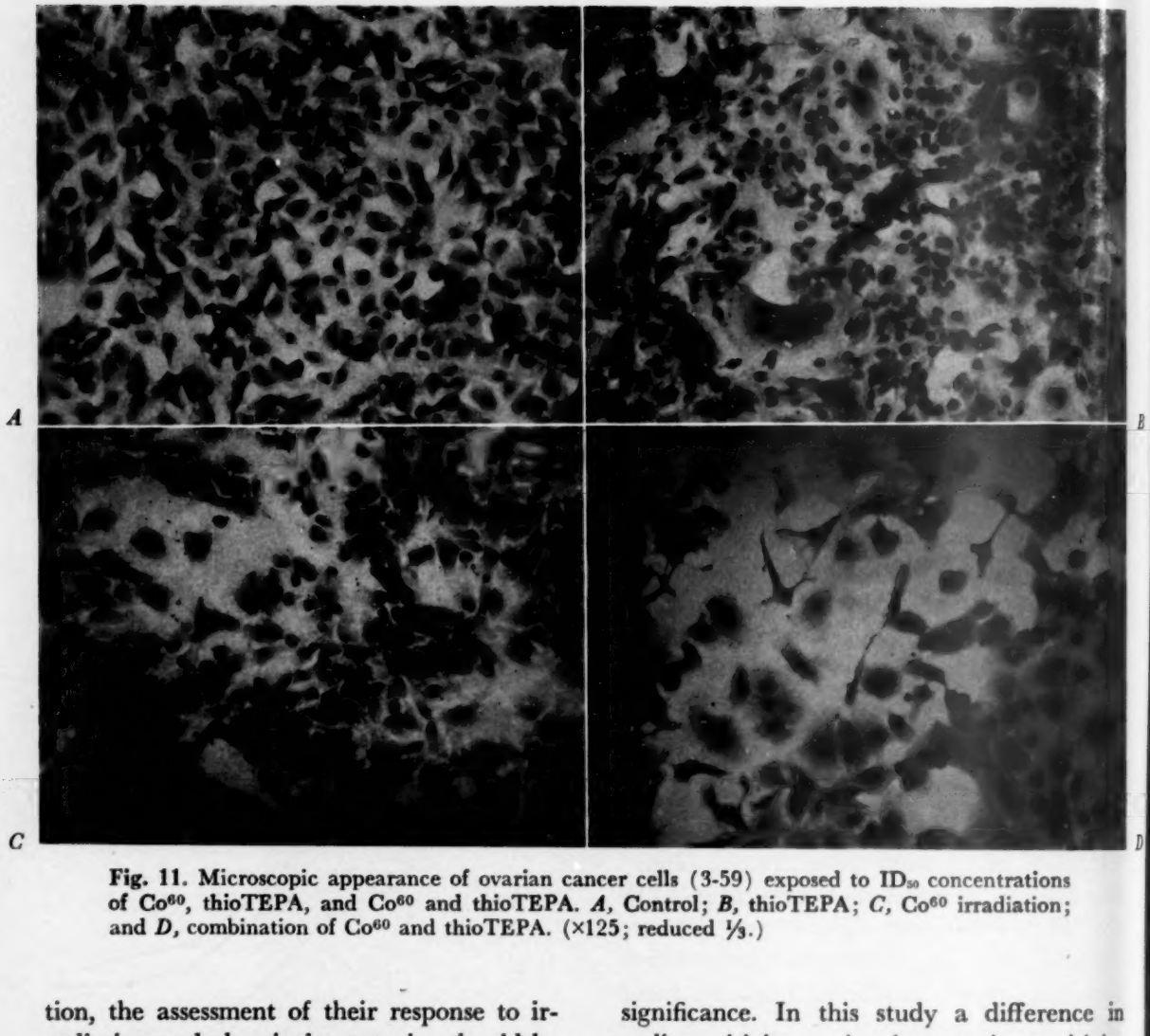


**Fig. 11.** Microscopic appearance of ovarian cancer cells (3-59) exposed to $ID_{50}$ concentrations of $Co^{60}$, thioTEPA, and $Co^{60}$ and thioTEPA. *A*, Control; *B*, thioTEPA; *C*, $Co^{60}$ irradiation; and *D*, combination of $Co^{60}$ and thioTEPA. (×125; reduced ⅓.)

tion, the assessment of their response to irradiation and chemical cytotoxins should be more clinically applicable.

In a broad sense, certain inferences from these studies can be made. It seems logical that if a cell strain develops tolerance to a cytotoxic compound in tissue culture, its precursors might also do so in the body. Less applicable would be the concept of the 50 per cent injurious dose ($ID_{50}$) determined in cell monolayers in tissue culture, if applied to three-dimensional neoplastic models growing within the protective limits of biological homeostasis.

Specific attention should be directed toward the aspects of this study which remain obscure. The time during the growth cycle of the cell culture in which it is subjected to the injurious agent may be of particular significance. In this study a difference in radiosensitivity or in chemotoxic sensitivity between the different cell strains could not be demonstrated. The fundamental work of Ludovici and associates[5] indicating that cell strains are more sensitive to irradiation suppression during the period of logarithmic growth (usually the second 24 hours) of the cell culture would appear to be of special significance. They feel that if the irradiation is employed during this phase of the cell culture's growth cycle their cell strains can be demonstrated to have different radiosensitivity responses, whereas during the less rapidly growing phases the several cell strains all appear to be equally radiosensitive. We are at the present time attempting to confirm this work with our own cell strains not only with regard to ionizing ir-

radiation but with alkylating agents. The selection of specific time for cytotoxic exposure during the sigmoid growth curves of these cultures may lend itself to a meaningful differentiation in response to chemotherapy as well as to irradiation between the several cell strains.

Varying the period of the growth cycle of the culture during which the cells are subjected to the injurious agent may also allow us to discern a difference between the effect irradiation and cytotoxic compounds have on cell population as opposed to cloning capacity. Under the conditions of the experiments herein reported, no differentiation between the two effects could be made.

**Summary**

A 12 to 22 hour period of exposure to thioTEPA was required in ovarian cancer cell cultures in order to exert a cytotoxic depression under the conditions of these experiments.

The development of tolerance to sublethal exposures of thioTEPA by ovarian cancer cells in vitro was demonstrated. A similar tolerance to sublethal exposures of ionizing irradiation by ovarian cancer cells could not be demonstrated.

The cytotoxic effect on ovarian and cervical cancer cells of $Co^{60}$ irradiation and combinations of $Co^{60}$ irradiation and thioTEPA was demonstrated in a quantitative manner. Under the conditions of these experiments the $ID_{50}$ dose of $Co^{60}$ was 200 r. Utilizing $ID_{50}$ doses of $Co^{60}$ and thioTEPA, no significant difference could be determined between the depressive effect of these injurious agents on cell population and their effect on cloning capacity of ovarian cancer cells (3-59) and cervical cancer cells (HeLa) in tissue culture.

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**Discussion**

Dr. James F. Nolan, Los Angeles, California. In the discussion of any presentation such as this, the question can always be raised as to whether the observed experimental results may be directly applicable to clinical situations. We tend to think of the biologic reactions related to the application of ionizing radiations (or to radiomimetic drugs) as combined responses of the tumor cells, of the tumor bed, and of the host in general. There is a natural hesitancy to accept observations of in vitro experimentation as being of therapeutic significance.

Dr. Moore emphasizes the limitations of tissue culture techniques from this point of view. His long and continued interest in the methodology, as well as his meticulous observations of the biologic behavior of cultured cells, points toward an ultimate goal of direct clinical application. His expressed intent is to qualify this method as one which will eventually allow for "sensitivity-testing" of fresh surgical material. Certainly, such a long-range attitude is laudable, but it would seem that the experimental data presented here cannot be entirely depreciated as having little significance. Actually, one can hardly study the reactions of tumor cells under stressful conditions which may be measured and reproduced without first placing them in controlled environmental situations.

In order to discuss Dr. Moore's most interesting contribution from this somewhat different point of view, one area seems especially intriguing. This concerns his observations of the re-

actions of standardized tissue cultures to an alkylating agent, as measured in terms of the relevant biologic effect described as their cloning capacity. The results, in comparison to the reactions of the same standardized cultures to ionizing radiation, show minor differences which are apparent in terms of a latency of effect, a "fixation response" related to time of exposure, and in the appearance of resistance to repeated sublethal exposures.

These differences in reaction seem paradoxic, since alkylating agents are considered to be radiomimetic. To be sure, radiomimetic drugs have the ability to simulate the biologic effects of ionizing radiations by definition. These biologic effects are considered to be: an interphase cellular death resulting in cellular disintegration before division; a mitotic cellular death resulting in cellular destruction at the time of the first few mitoses; and mutogenic and cancerogenic effects.

It is considered that alkylating agents may act through many intermediate chemical reactions, but that their ultimate effect is to cause a "cross-linkage" of DNA molecules which eventually results in an unnatural configuration of the genetic material. This reaction leads to difficulties when DNA molecules must be shared between daughter cells. Although ionizing radiations can cause such "cross-linkage" of DNA this effect is not considered to be the primary one. There are many intracellular mechanisms which may be disturbed by the chemical intermediaries which result from physical ionization. It has been suggested that the sequence of events initiated by alkylating agents and by radiation may follow somewhat different paths and meet only at the stage of detectable biologic disturbances. Thus, it may be theorized that the explanation for the differences in results reported here are due to the prominence of the "mitotic death" type of reaction from exposure to the alkylating agent as compared to the prominence of the "interphase death" response from exposure to radiation.

Although such an explanation as this may be entirely speculative, the fact that demonstrable differences in reaction did appear promotes further speculation. One wonders if knowledge of these basic facts could not improve our understanding of the concepts of augmentation and enhancement of radiation reactions. The obvious example of utilization of such information is in the improvement of our present methods of combined radiotherapy and chemotherapy. The clinical application of improved treatment methods to such problems as the management of advanced ovarian cancer remains a pressing need.

Dr. Moore (Closing). I should like to thank Dr. Nolan for the job he did in discussing this paper. He not only went over it meticulously and made very pertinent suggestions for work that should be done, but he came out to the laboratory and helped set up the experiment that would implement his suggestions. He brought out the fact that it is paradoxical that we found differences in the reactions in these cells and tissue cultures with alkylating agents and ionizing irradiation. I do not think this is paradoxical. The end results seem much the same but there is a tremendous difference in timing. I only wish that Dr. Norman Miller had had a chance to discuss some of the differences he found in timing the ionizing irradiation as it was applied in different stages in the culture of his cells in tissue culture. He found the most effective time to apply irradiation was during the second 24 hour period of the culture. Those of you who are acquainted with tissue culture know this is the stage of most rapid growth and it would be logical that this would be the time when ionizing irradiation would be most effective. However, we found that in alkylating agents the most effective time application was in the first 24 hours, that is, during the period of the first generation of the cells. I wish that I had had the foresight to do as Dr. Miller did and give radiation at other periods during the growth of the cultures. We gave it after seeding cells in the culture and this may not be the most effective time.

There are other indications that timing plays a part in producing this difference between alkylating agents and ionizing irradiation. Fig. 10 illustrated the fact that irradiation effects took place more quickly than the effect of alkylating agents. This I think should be studied much more intensively, and in this regard Dr. Nolan has made pertinent suggestions. I hope that on another occasion we can come back with the answer to these problems. Again I would like to thank Dr. Nolan for his well-placed comments.

# Dose distribution during the radium therapy of cervical cancer

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THE effect of radium therapy upon malignant and normal tissues is a function of the radiation dose. In radiation therapy for cervical cancer, it is obvious that the best management requires an estimate of dose in specific pelvic structures.

Dose rates for one or two points or planes about an applicator are often precalculated from the known dimensions and loading of the applicator. This is useful when considering the merits of different applicators, but it yields only very limited information about the actual dose delivered to a specific organ in a given patient. If such a drawing board approach is used to control the therapy of individual patients, the therapist may be lulled into the false belief that dose rates for pelvic tissues are consistent from patient to patient for the same applicator and specified loading.

Much more detailed information is required for adequate control of the radium therapy. Orthographic radiography is a method which provides information regarding exposure dose rates for radium at an infinite number of points throughout the pelvis. The practical application of this technique at the Hospital of the University of Pennsylvania has permitted individualized determination of the exposure dose rates at specified points within the parametrium and vital organs and it has shown that these dose rates vary widely. It is the purpose of this report to describe some of these variations and to point out their relationships to clinical observations.

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## Materials and methods

During the period from April 1, 1952, to Aug. 31, 1958, 236 patients with malignancy involving the cervix were treated with radium at the Hospital of the University of Pennsylvania. Twenty-four patients were excluded from this analysis because of a total or supravaginal hysterectomy prior to irradiation or because the bony pelvis was inadequately demonstrated on postapplication roentgenograms. The 212 patients who constitute the basis for this report had dose control through the use of orthographic roentgenography according to a technique previously reported.[1, 2] In 10 instances, unsatisfactory distribution of radiation required prompt removal and reapplication of the radium. Studies of the second application for these 10 patients have been omitted from this report.

The age and parity distribution for the 212 patients is given in Tables I and II. Six

**Table I.** Number of patients according to age, by decades

| *Age* | *No.* |
|---|---|
| 20 to 29 | 7 |
| 30 to 39 | 40 |
| 40 to 49 | 53 |
| 50 to 59 | 61 |
| 60 to 69 | 30 |
| 70 plus | 21 |
| Total | 212 |

**Table II.** Number of patients according to parity

| *Parity* | *No.* |
|---|---|
| 0 | 27 |
| i | 46 |
| ii | 44 |
| iii | 23 |
| iv | 19 |
| v plus | 53 |
| Total | 212 |

**Table III.** International Stage of patients with primary cervical cancer

| *Stage* | *No.* |
|---|---|
| 0 | 3 |
| I | 96 |
| II | 70 |
| III | 27 |
| IV | 5 |
| V | 5 |
| Total | 206 |

of the patients had adenocarcinoma involving the corpus and cervix. The remaining patients had primary cervical malignancy, 197 squamous cell carcinoma, 4 adenocarcinoma, 3 adenoacanthoma, 1 mesonephric-type carcinoma and one both squamous cell carcinoma and adenocarcinoma. The International Stages for these patients are shown in Table III. The frequency of involvement of various local sites by cancer which had spread beyond the cervix as determined by clinical evaluation is given in Table IV.

The Ernst applicator[3] and a modified Manchester applicator[2] were the only radium containers that were utilized (Table V).

Containers were classed as rigid when the component parts were firmly interlocked and nonrigid when the parts were loosely related to one another. The first 35 Manchester-type insertions were nonrigid. The applicator was then modified by the use of a metal intrauterine stem which was firmly attached to the spacer.[2] Despite the modification, displacement of the spacer or ovoid occurred in 13 instances. These were included in the study of nonrigid containers.

When the vaginal vault was too small to permit the introduction of the smallest Manchester units, the Ernst applicator was used. The Ernst applicator was inserted with only 4 vaginal radium holders. Each was loaded with 10 mg. of radium filtered by the equivalent of 1.5 mm. of platinum. The lateral detachable holders were never employed. The intracervical and intrauterine stem measured either 2 or 3 holders in length. The most inferior unit was always unloaded. The next superior holder was loaded with 20 or 25 mg. of radium (filter equivalent to 0.5 mm. of platinum). The third unit, when employed, always contained 10 mg. of radium (filter equivalent to 1.0 mm. of platinum).

When the Manchester applicator was employed, the choice of size of the ovoids and spacer was based upon the size of the vaginal vault. Table VI indicates the number of insertions that occurred on the basis of the combined transverse widths of the vaginal components (measured from ovoid center to ovoid center) utilized for each insertion. In 4 patients, ovoids of unequal size were placed in the vaginal fornices. The Manchester-type applicator was loaded according to a unit system: 3 cm. diameter ovoid, 5 units; 2.5 cm., 4 units; 2.0 cm., 3 units. The stem loading varied between two basic patterns; for 136 patients 1, 2, 1 units, and for 42 patients 2, 2, 2 units. The radium unit was 10 mg. for patients treated with orthovoltage x-ray in conjunction with radium therapy. The radium unit was 5 mg. for the last 14 patients in the series, all of whom received supervoltage x-ray therapy.

The procedure for the insertion of ra-

dium, the radiograph exposure, the correction of distortion, and the calculation of actual dose rates at Points A and B of Tod and Meredith[4] followed the technique described previously.[2] The placement of radiopaque catheters in the ureters permitted determination of the course of the ureters 205 times for the left side and 204 times for the right. Radiopaque media in the bladder permitted adequate visualization of the bladder for 204 patients. The rectum and rectosigmoid were adequately visualized for all patients with the aid of a barium sulfate suspension.

Examination of the radiographs and the pantograph drawings permitted evaluation of the spatial relationships of the radium to the vital pelvic organs and selection of those points in the mucosal surfaces of the organs which were subject to the highest exposure dose rates. These rates in roentgens per hour (expressed in the nearest whole number) for points of interest were determined by the position of these points with respect to each radium container. This procedure was readily applicable to most of the patients. In a few cases it was necessary to calculate exposure dose rates for several tissue zones in order to be certain of the choice of the highest dose points.

As indicated previously, the radium loading varied from patient to patient. Therefore, a common denominator was needed for the purpose of comparing the variations in dose distribution. This was obtained by the use of the actual exposure dose rate at right Point A. For each patient this value was divided into the exposure dose rates for the other points of interest (Table VII). These ratios were defined as the relative exposure dose rates. Selected portions of the results were checked by the use of left Point A as a standard reference. The results were essentially the same as for right Point A. Therefore, relative exposure dose rates are reported only with right Point A as a denominator.

The data for the entire series of 212 patients were studied for interrelationships of clinical observations, relations of clinical findings to relative exposure dose rates, and correlation of relative exposure dose rates.

All data regarding clinical observations and dose rates were transferred to business machine cards. The distribution and relationships of results were obtained by machine card selection and correlation.

**Table IV.** Frequency of clinical involvement by cancer of sites beyond the cervix

| *Site* | *No. involved* |
|---|---|
| Rectovaginal septum | 22 |
| Vesicovaginal septum | 18 |
| Lateral fornix | 46 |
| Right parametrium, partial | 40 |
| Left parametrium, partial | 36 |
| Right parametrium, complete | 20 |
| Left parametrium, complete | 28 |
| Endometrium | 18 |
| Beyond above sites | 7 |

**Table V.** Frequency of insertions of Ernst and Manchester-type radium containers

| *Type* | *No.* |
|---|---|
| Ernst | 34 |
| Manchester-rigid intact | 130 |
| Displaced ovoid | 13 |
| Nonrigid | 35 |
| Total | 212 |

**Table VI.** Number of radium insertions, Manchester-type, based on distance between ovoid centers

| *Transverse width (cm.)* | *No. of insertions* |
|---|---|
| 3.0 | 44 |
| 3.5 | 51 |
| 4.0 | 53 |
| 4.5 | 26 |

## Results

Table VIII shows the location of the highest exposure dose points within the pelvic organs. The sites of greatest dosage were essentially the same for most of the patients. In all but one of the patients, the major portion of the highest exposure dose rates for the bladder, rectum, and ureters was pro-

**Table VII.** Determination of relative exposure dose rates in a representative case

| | *Site of exposure* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *A* | | *B* | | | *Ureter* | | |
| | *Right* | *Left* | *Right* | *Left* | *Bladder* | *Right* | *Left* | *Rectum* |
| Actual exposure dose rate (r/hr.) | 74 | 73 | 29 | 32 | 60 | 100 | 76 | 63 |
| Relative exposure dose rate (values first line divided by 74) | 1 | 0.99 | 0.39 | 0.43 | 0.81 | 1.35 | 1.03 | 0.85 |

**Table VIII.** Location of points of highest dose rate for vital pelvic structures

| *Organ* | *No. visualized* | *Point location* | *No.* | *%* |
|---|---|---|---|---|
| Bladder | 204 | Mid-posterior | 50 | 25 |
| | | Lateral posterior | 154 | 75 |
| Rectum | 212 | Posterior to cervix | 187 | 88 |
| Rectosigmoid | | Posterior to uterus | 16 | 8 |
| | | Near uterine fundus | 9 | 4 |
| Right ureter | 204 | Parametrium | 154 | 75 |
| | | within bladder wall | 50 | 25 |
| Left ureter | 205 | Parametrium | 171 | 84 |
| | | within bladder wall | 34 | 16 |

**Table IX.** Relative exposure dose rates

| *Site* | *No.* | *Range* | | *50% Range** | | *Mean* | *Standard deviation†* |
|---|---|---|---|---|---|---|---|
| | | *From* | *To* | *From* | *To* | | |
| Left Point A rigid | 164 | 0.86 | 1.11 | 1.00 | 1.00 | 1.00 | ±0.08 |
| Left Point A nonrigid | 48 | 0.60 | 1.64 | 0.89 | 1.09 | 0.97 | ±0.19 |
| Right Point B | 212 | 0.15 | 0.75 | 0.30 | 0.43 | 0.36 | ±0.10 |
| Left Point B | 212 | 0.18 | 0.68 | 0.30 | 0.41 | 0.35 | ±0.09 |
| Bladder | 204 | 0.34 | 2.94 | 0.77 | 1.23 | 1.00 | ±0.40 |
| Right ureter | 204 | 0.44 | 2.00 | 0.82 | 1.28 | 1.05 | ±0.32 |
| Left ureter | 205 | 0.22 | 2.15 | 0.78 | 1.10 | 0.92 | ±0.29 |
| Rectum | 212 | 0.38 | 2.10 | 0.75 | 1.05 | 0.90 | ±0.28 |

*Range which includes 50% of patients.

†Standard deviation of single values. All of the distributions except left Point A (rigid applicators) were essentially normal, as shown by linear cumulative plots on probability paper.

vided by the intravaginal radium. In the one exception, a right ureter, slightly more than half of the highest exposure dose was contributed by a stem within a uterus with marked lateral version.

Table IX summarizes the highest relative exposure dose rates for the Points A and B and organs (bladder, ureters, and rectum) studied.

The distribution of relative exposure dose for left Point A confirmed the observation previously reported that the left to right Point A dose rate ratio is frequently 1.00 when a rigid applicator is used but varies widely when nonrigid applicators are used. For 79 per cent of rigid applicator insertions, the left Point A relative exposure rate was 1.00 ± 0.03. The value ± 3 per cent has been shown to represent the limit of accuracy of the orthographic technique.[1] The other 21 per cent of the rigid insertions had relative exposure dose rates at left Point A in the range of 0.86 to 0.96 or 1.04 to 1.11. In three fourths of these, the applicators were

found to be rotated 5 or more degrees with respect to the long axis of the patient's body, a condition observed in only 5 per cent of the other 130 rigid insertions. Since Point A by definition must remain directly lateral to the uterine axis, rotation of the applicator changes the relation of the vaginal radium to Point A. In the remaining one fourth of the rigid insertions the variation was apparently due to eccentric placement of radium in the ovoids.

In contrast, a much wider range of values was noted for the nonrigid units. Only 21 per cent of the values were within the range $1.00 \pm 0.03$.

The large difference between rigid and nonrigid applications was seen only in the Point A relative dose rates. For the other sites, therefore, the rigid and nonrigid applicator data are shown as pooled in Table IX.

### Clinical factors interrelated

Prior to the analysis of the correlation of clinical observations with relative exposure dose rates, the interrelationships of clinical observations with each other were studied for the 179 cases whose data were complete in all particulars. The remaining patients were excluded because the ureter or bladder was not visualized (19 cases), the malignancy could not be staged as International I to IV (11 cases), or the ovoids were of unequal size (4 cases).

It was found that the older patients tended to have higher stage classifications, as shown in Fig. 1. This correlation was computed by standard regression methods,[5] after dividing the patients into three age groups (less than 40, 40 to 59, and more than 59) and three groups of International Stage (0 and I; II; III and IV). It is statistically significant ($p < 0.02$) that true regression coefficient is zero.

The radium applicator used provides a measure of the vaginal size or distensibility, since in nearly all cases the largest applicator that could be inserted was employed. The four Manchester combinations cover a range of transverse widths as stated in Table V. The Ernst applications were considered as one step more narrow, since it was generally found that the Ernst applicator could be used when the vagina was too small to admit the smallest Manchester units. Fig. 1 shows a strong dependence of vaginal size upon age, which was statistically highly significant ($p < 0.001$).

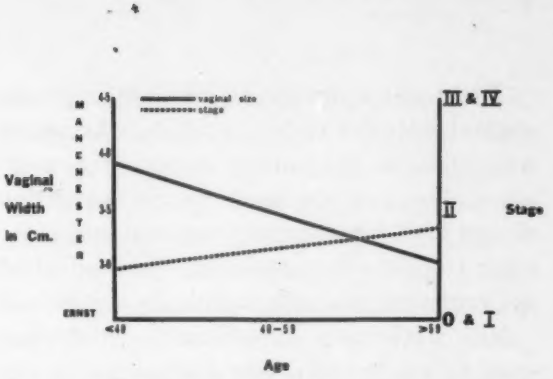


Fig. 1. Variation of Stage with age and vaginal size with age. In this series, vaginal width diminished and International Stage increased with advancing age.

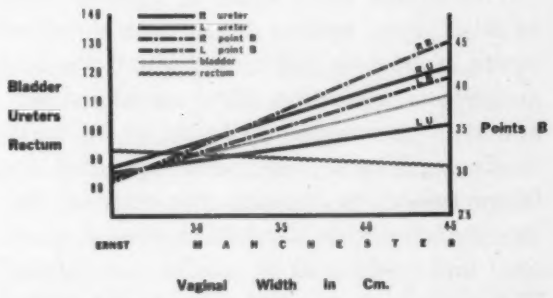


Fig. 2. Variations of relative exposure dose rate with vaginal size as measured by the width of the vaginal radium applicator. The increase in relative dose for all points (except rectum) with increasing vaginal width is statistically significant.

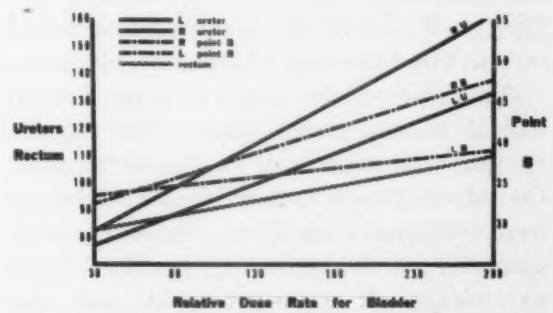


Fig. 3. Correlation of bladder relative dose rate with rates at other points within the same patient. There is a concomitant variation in rates for all points.

There was a correlation between stage and vaginal size, the wider applicators being associated with the earlier stages. However, when allowance was made for the association of age with both vaginal size and stage, the stage–vaginal size correlation (with effect of age removed) was not statistically significant.

No statistically significant correlations were found between age and parity or site of tumor involvement (Table IV) or between stage and these factors.

### Dose related to clinical factors

The effects upon relative exposure dose rates of age, parity, stage, clinical tumor extent, applicator width, applicator position, and Point A location were considered separately. The correlations found to be statistically significant were then corrected for interrelationships between the various factors, by computing standard regression equations and coefficients of partial correlation.[5] This gives a measure of the correlation between two variables when the effect of a third variable (having a known correlation with each of them) is removed. The probability that the degree of correlation observed could be explained by random sampling error is then obtained from statistical tables. The following results were obtained:

**Age.** The relative exposure dose rates at several of the points were higher in the younger patients than in the older ones. Considered by themselves, these correlations were statistically significant. However, when corrected for the previously described interdependence of applicator width and age, none of the age-dose relationships were significant.

**Parity.** There were no significant variations in relative exposure dose rates associated with parity.

**Stage.** The relative dose rates at all points appeared to be slightly lower in the more advanced stages, but when the correction for correlation of stage and age was applied, the correlations between dose and stage (independent of age) were found to be not statistically significant at any point.

**Clinical tumor extent.** There appeared to be no consistent relationship between the sites involved by tumor (Table IV) and the relative exposure dose rate.

**Applicator width.** The relative dose at every point, except the rectum and left Point A, showed significant correlation with the applicator width (Fig. 2). The analysis of partial correlation showed that this could not be attributed to the interdependecy of age and applicator width ($p < 0.01$ with the effect of age removed). However, analysis by this method did show that the correlations of applicator width with bladder dose and left ureter dose were not significant when the effect of the very close association between the dose rates at those structures and the right ureter dose was removed.

**Applicator position.** The relative anterior-to-posterior location of the intrauterine stem was studied with respect to its possible effect upon relative exposure dose rates. The angle between the stem and the mid-coronal plane of the pelvis served as the basis for establishing relative anterior or posterior positions. When the angle was more than 10 degrees and the direction of the stem was anterior to the coronal plane, the dose rates for both Points B, bladder, and both ureters were slightly higher than when the stem-coronal plane angle was 10 degrees or less.

Rotation of the entire applicator about the long axis of the patient's body was considered for two groups of patients: (1) those with less than 10 degrees and (2) those with 10 degrees or more of rotation. The latter degree of rotation appeared to affect only the bladder rate (14 per cent increase in mean value).

**Right Point A location.** In a previous publication, the precise location of Point A was described with respect to specific anatomic landmarks.[2] With the same techniques, right Point A was located with respect to the interacetabular line and the mid-sagittal plane for all of the 212 patients in this series.

The location of right Point A with respect to the line and the plane was not uniformly associated with any effect upon the relative

exposure dose rates, except that, as might be anticipated, the relative rate at Point B had for the right side a direct relationship and for the left side an inverse relationship to the distance from right Point A to the mid-sagittal plane.

### Relative rate correlation

In order to determine whether a low, intermediate, or high dose rate at one point was likely to be associated with a similar level of dose at other sites, the relative exposure dose rates for the bladder were used as a basis for comparison. This organ was selected since it was observed to have the greatest range of relative dose rates. The correlation of bladder dose rates with rates at other sites within an individual is shown in Fig. 3. It was found that, as the bladder relative exposure dose rate increased from lowest to highest value, there was an associated increase in values for right and left ureter, the rectum, and the right and left Point B. All of these correlations were statistically significant with the exception of left Point B.

The general trend of Fig. 3 was anticipated, since the patients in whom large applicators were used would be expected to show higher relative dose rates at all points distal to Point A than would those with smaller applicators (see Comment). However, even when the influence of applicator size was removed from these correlations, the bladder dose still showed highly significant correlations with both ureters ($p < 0.01$) and correlations of borderline significance with right Point B and the rectum ($p \approx 0.05$).

### Comment

The most important result of this study was the demonstration of wide individual variations in both the actual and relative exposure dose rates for Points A and B and the vital pelvic organs. For the entire series, the mean values of exposure dose rates for each of the vital pelvic organs were very close to or equal to the mean values for both Points A. However, the exposure dose rates for bladder, ureters, and rectum, when contrasted to the individual patient's right Point A exposure dose rates, varied over such a wide range that the mean value relationships appear to be clinically useless.

The marked differences in individual relative dose rates were noted despite the fact that in 75 per cent or more of the patients, the highest exposure dose rates were located in the same general regions of the bladder, ureters, and rectum. It was apparent that, although a therapist might expect the same spatial distribution of irradiation, at least about a rigid applicator, no consistent relationship of organ dose rates should be anticipated. Only Point A dose appears to be consistent from patient to patient, presumably because the location of this point is essentially determined by the position of the radium. All other points will depend upon the anatomic variations of the individual patient.

The only statistically significant correlation between clinical observations and relative exposure dose rates, after account was taken of the interdependence of some of the clinical factors, was the increasing dose rates with increasing vaginal applicator widths. This correlation was highly significant for both Points B and the right ureter. For the bladder and left ureter this correlation appeared to be mostly a consequence of the general consistency of bladder and ureter doses within individuals. For the rectum there was no correlation with vaginal width. Thus it appears that even the most obvious theoretic generalization, namely, that those patients who can accommodate larger vaginal radium containers will be subject to larger relative exposure dose rates at all points beyond Point A, is not completely verified in actual practice. For this reason it seems unlikely that meaningful estimates of dose to the bladder, ureters, and rectum can be made for the individual patient on the basis of drawing board precalculations of the dose distribution around a particular type of applicator.

The mean exposure dose rate for the left ureter was substantially lower than for the

right. The left ureter was noted in the majority of cases to follow a different course through the parametrium and bladder wall from the right ureter. On the right side the ureter frequently appeared to be parallel to the isodose surfaces about the anterior half of the vaginal radium. In contrast, the left ureter approached the vaginal radium along a course which was approximately perpendicular to the anterior end of the vaginal radium. It is felt that this difference in the courses followed by the ureters may account for the lower mean relative dose on the left side and the lack of correlation with applicator size.

The relative rectal dose rates appeared to be unrelated to any clinical observation, either singly or combined. The rectal dose is dependent upon the space between rectal mucosa and vaginal radium containers and the dose rate distribution about the vaginal radium. The width of this space appears to have varied in a random manner despite the fact that the operator always attempted to place 1 to 1½ cm. of packing between the vaginal containers and the rectovaginal septum.

Low, intermediate, or high bladder dose rates were statistically correlated with similar levels for other sites except left Point B. These relationships were attributed in part to the dose distribution about containers. The dose rates in the tissues about the Ernst as used in this series and the smallest vaginal Manchester units decrease with relative rapidity as the distance from the applicator increases. There is not so rapid a decrease in exposure dose rates about the larger vaginal Manchester units. Thus, there is greater opportunity for all the dose rate points to receive low exposures to irradiation for the Ernst applicator or smallest vaginal Manchester units. In contrast, the same exposure dose points, when irradiated by a larger vaginal Manchester applicator, will all tend to have relatively high exposure dose rates.

Not all of the correlation between the various dose rate points in the same patient could be explained by applicator size. There appeared to be some feature of the patient other than the factors analyzed here, which resulted in a tendency for those patients having low relative dose rates at the bladder to also have low relative dose rates at the other points and vice versa. This association was particularly striking for the bladder and ureters. It may be speculated that individual variations in pelvic anatomy might account for this relationship.

Such applicator considerations as rigidity, rotation, or angulation were not found to have significant effects upon the mean relative dose rates.

A contrast of Ernst and Manchester units could not be made because there was a specific selection procedure followed in the use of applicators, the Ernst applicator being employed only for patients with the smallest vaginas.

## Summary

The radium applications for 212 patients with cancer of the uterine cervix were studied with regard to dose distribution. The right Point A exposure dose rate for each patient served as a standard reference with relation to dose rates at left Point A, both Points B, and the highest exposure dose rates in the bladder, ureters, and rectum. Although the highest dose rates were found in the same general regions of the pelvic structures, there was a wide variation from patient to patient. The only consistent relationship found was between left Point A and right Point A, for rigid applicators only. Age, parity, stage, and tumor extent did not appear to influence relative dose rates significantly, but there was a highly significant correlation with the size of the vaginal vault, as it determined the type and size of radium applicator. There was also a direct correlation between relative dose rates at the various points in the same patient, indicating an effect of individual anatomic characteristics.

## Conclusions

It must be concluded, on the basis of this study, that the major factor which deter-

mines the relative dose rate for parametrial points and the vital pelvic structures is the individual anatomic relationship of the point or structure to the radium containers; these relationships vary widely between patients. Therefore, dose rates for a given patient cannot be predicted. Precalculated dose rates at points or in planes about an applicator do not reliably estimate the exposure dose rates the pelvic organs receive. Accurate determination of radium dose rates requires a technique that takes into account the individual anatomic variations for each application of radium.

Appreciation is expressed for the advice, encouragement, and assistance of Dr. Franklin L. Payne, Chairman of the Department of Obstetrics and Gynecology, and Dr. Richard H. Chamberlain, Chairman of the Department of Radiology, School of Medicine, University of Pennsylvania.

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## Discussion

DR. JOHN L. MCKELVEY, Minneapolis, Minnesota. This work by Drs. Lewis and Raventos is a significant contribution to the accuracy of radium therapy of pelvic lesions. Most of the conclusions which they have drawn were already known but their demonstration of the degree of variation which may occur under circumstances which may not be readily recognizable will warn us all again.

Those of us who have read the previous papers by them will realize the amount of work which has gone into developing and applying the techniques which they use for measuring dosages at various areas within the pelvis. The technical discussion of their procedure will have to be left to Dr. Arneson. One can only agree with them that the use of a theoretical Point A and Point B may be comforting to those who use them but are so overlaid by anatomic variations as to be often misleading. Under any circumstances, they are not necessarily the areas of real importance in therapy. They are, however, points which can be used to demonstrate roughly variations from calculated dosage when anatomic variations or deviations from the hoped for positioning of radium containers have occurred. When nonrigid applicators were used, the variations in dosage in 80 per cent of the patients at the two Points A were from 60 to 165 per cent, which is startling.

The technique of measurement which they describe is an accurate investigational tool but is rather complicated for general use unless one can demonstrate the necessity for complete accuracy of dosage at distance. Even with this technique, it is impossible to achieve accuracy without the use of rigid applicators which fix the spatial relation of the intrauterine, intracervical, and vaginal sources. One cannot avoid the conclusion that there are advantages to the use of nonrigid applicators. Under these circumstances, we compensate for the inaccuracies by using dosages and distributions of sources which have been clinically demonstrated to be reasonably safe and clinically productive. One also relys upon the fact that, within these ranges, the organs most subject to trauma have a considerable range of dosage tolerance. The technique used at Minnesota involves a total dose from x-ray or cobalt and radium from a standard 5 portal setup of about 7,000 r to the posterior wall of the bladder and the anterior wall of the rectum. This is well beyond the theoretical damaging dose, but these organs almost always recover at least clinically if not entirely histologically. Information has been collected by direct measurement of the variations in dosage and of the natural history of the changes in the rectum and similar work is now in progress to study the bladder. There is little evidence that variation in even such large dosage leads to significant pathologic conditions. There has been, for practical purposes, no trouble with the ureters. For

these reasons, complicated individual patient dosage measurement has not been attractive.

If there were reason to believe that pushing the radium dosage beyond the presently generally used levels would add to the cure rates, then accurate control by some such technique as they advocate would be a sine qua non. There does not seem to be such an indication. The local tumor in the cervix and the proximal parametrium are presently being overirradiated in order, with a short focal distance source, to achieve what the Germans call a *zusatz dosis* to the pelvic wall area. The main reliance for dosage to all but the cervix and adjacent parametrium must be on the long focal distance irradiation source and here one is protected by the inverse square law. Even with long focal distance therapy, dosage at depth varies from the calculated dose and often considerably. By direct measurement in the cervix, we have seen as much as a 25 per cent variation from the calculated x-ray dose.

Altogether then, one is inevitably dealing with a dangerous series of variables which are not as accurately controllable as one would earnestly wish. It is this which commends their struggles to remove some of these. Certainly if one is to work without such safeguards, he must be thoroughly familiar with at least accurate compound isodose curves for distribution of dosage from the various combinations of sources which anatomic variations may dictate and he should have sufficient experience and careful long-time observation of results to be assured of both the adequacy and safety of his techniques. Most of us will have to depend on this less accurate control and it is a worrisome game.

Dr. A. N. Arneson, St. Louis, Missouri. The presentation by Dr. Lewis is founded upon an extensive experience in radiation dosimetry accumulated with Dr. Raventos over a span of several years. In studying the distribution of radiation in the radium treatment of cervical cancer, they have traced the wide and irregular variations in dose level throughout the pelvis. Irregularity is inescapable because the volume to be exposed must be irradiated by centrally located sources. The anatomic difficulties encountered in this area are not resolved by a more diffuse distribution of radium applied interstitially by needles introduced into the parametrial regions.

The irregularity in dose level is greater in the zone immediately adjacent to the central radium setup. It tends to disappear in the more lateral regions of the pelvis. This is due to the rapid fall-off in intensity at points nearer radium sources, but with modest increase in distance, the changes per unit of distance become significantly less. Mindful of the irregularities here involved, a more representative reference to the whole dose is gained by specification of amounts at certain selected points with spatial relationship. General acceptance has been given the theoretical points designated as "A" and "B," developed in the Manchester system of radium dosage and intentionally located lateral to the uterus in the normal pathway of spread for cervical cancer. The relative dose value of "B" is stated to range from 0.2 to 0.4 of that at "A." Dr. Lewis and Dr. Raventos have confirmed that relationship but in their investigations they have developed a more meticulous study of dose distribution in the zone of greater irregularity immediately around the radium sources. The values for different points in that zone are given in reference to the dose calculated for "A." Approximately 75 per cent of all high levels occurred in the same general area, namely, bladder, rectum, and the ureters. Those are critical structures subject to injury.

Upon investigating the cause for overirradiation at some points, they found the size of vaginal ovoid to be a significant factor. The differential loading in the Manchester system is aimed at providing a uniform intensity at the surface of the vaginal applicator regardless of actual size. The larger sizes must, by necessity, be loaded with greater amounts of radium to compensate for the increase in radial distance. The increased distance affects depth dose in adjacent tissues by a somewhat lesser rate of fall-off in intensity of radiation. As a consequence there is risk of higher dose levels at some points. The situation is, however, not entirely correctable by change in applicator size because, as implied by Dr. Lewis, anatomic variation in different patients appears to be a responsible factor.

One of the significant results in these researches is the emphasis given the fact that Points "A" and "B" are not by themselves of critical importance. Any standardization of procedure aimed only at accumulating a specified dose at "A" becomes a method aimed at treating theoretical points rather than living patients. This does not lessen the importance of recording dose in reference to those points, but, as

Dr. Lewis has pointed out, it is not sufficient to apply radium, consult an appropriate dosage table, and calculate exposure time on the basis of data for Point "A." His technique of meticulous dose assessment by radiographic study appears too complex for routine use. It belongs in the area of research. In every instance, however, the radium setup should be at least viewed in radiographs and each application should be monitored by some apparatus such as the scintillation counter.

Finally, there is a more philosophic point to be mentioned in conjunction with this presentation. The excellent collaboration between gynecologist and radiation therapist at the hospital of the University of Pennsylvania speaks for itself. The radiation management of cervical cancer has become too complex for administration by one individual. The overwhelming details of dosimetry cause a drift of responsibility into radiology. That is, however, only one facet of the whole problem, but rightful continuity of gynecology in the total picture depends upon availability of other specialists with interests and talents similar to those of Dr. Lewis.

Dr. Lewis (Closing). I admit that this study is complex but there are being developed several methods for simplifying the system so that it can be used more broadly. There are some other ways of doing this which will make it easier to employ dosage distribution checkup during therapy.

# Treatment of cancer of the cervix by radiation and elective radical hysterectomy

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SINCE our initial report on Stage I lesions, there have been many papers describing results of combined radiation and elective radical operations for cervical carcinoma.[2, 6, 8] Many have been preliminary reports, all anticipate improved survival, but few permit long-term evaluation of the method or comparison with a control group. Including the previously reported experience with Stage I lesions,[7] a group of 101 patients with International Stage I, II, and III cancer treated with conventional radiotherapy alone and a similar group of 93 patients who received additional elective radical hysterectomy and pelvic lymphadenectomy have been followed at the North Carolina Baptist Hospital (NCBH). With few exceptions, all patients are 5 years post therapy and many are surviving for as long as 10 to 15 years. The patients have been followed predominantly at the NCBH Clinics and in some cases by their family physicians. Consultations were made whenever the question of possible recurrence arose. When deaths from intercurrent disease occurred, persistent tumor was excluded by postmortem examination or, if this was impossible, survival over 5 years with recent negative examination for cancer was required before the patient was categorized in a survival group. One patient was completely lost to follow-up study 8 years after treatment, at which time there was no evidence of recurrent tumor.

It is the purpose of this paper to analyze statistically survival in the two therapy groups. Differences in the patient content of each group and in therapy complications will also be discussed as essential data for a proper interpretation of the results.

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### Stage II and Stage III experience

One hundred and twenty-six Stage II and 50 Stage III patients were treated primarily at the NCBH from Jan. 1, 1943, to Dec. 31, 1958. Respectively, they represent 32.6 per cent and 12.9 per cent of the 387 patients diagnosed and treated during this period. Seven women (4 Stage II and 3 Stage III) had stump carcinoma. Diagnostic procedure, pretherapeutic evaluation, and variations of treatment followed that described for Stage I patients.[7] "Standard radiation therapy" will be used to describe radiation levels delivered by conventional means as outlined in Table I and should be distinguished from "intensive radiotherapy."

The patients fall into five distinct therapeutic categories: Group I, standard radiation therapy alone; Group II, combined standard radiation and elective radical operation; Group III, intensive radiotherapy;

Group IV, primary radical operation (no patients were so treated in this series); Group V, miscellaneous therapy; and Group VI, carcinoma of the cervical stump. The substage distribution and histology of the lesions and the socioeconomic status and age distribution of the patients are given in Table II. Survival after therapy and complications of therapy are listed in Tables III and IV. Only severe complications of treatment have been considered. A detailed discussion of complications following intensive radiotherapy was presented previously.[5]

Results of miscellaneous therapy and therapy for stump carcinoma generally parallel those noted for Stage I patients. When radiation therapy was compromised, supplemental radical operation has consistently given rewarding survival. The results after intensive radiotherapy have been included for completeness and to emphasize the intolerably high complication rates, especially when additional radical operation was performed.

### Analysis of surgical pathology

The sites and incidence of residual carcinoma after standard radiation therapy and

**Table I.** Radiation dosimetry

| | *Cervix* | *Point A* | *Point W* | *Bladder and rectum* |
|---|---|---|---|---|
| Standard radiation (194 patients) | 11,600 r* (7,200-17,800) | 7,200 r* (6,000-9,300) | 3,600 r* (2,900-5,300) | — |
| Intensive radiation (55 patients) | 10,850 r (6,000-15,800) | 9,350 r (6,000-13,400) | 5,100 r (3,000-7,400) | 5,700 r (3,400-9,100) |

*Total of intracavitary gamma roentgens and external radiation.

**Table II.** Distribution of variables in patients with intact uteri

| | *Stage II* | | | | | *Stage III* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Entire stage (122 patients) (%)* | *Group I (48 patients) (%)* | *Group II (33 patients) (%)* | *Group III (21 patients) (%)* | *Group V (20 patients) (%)* | *Entire stage (47 patients) (%)* | *Group I (15 patients) (%)* | *Group II (10 patients) (%)* | *Group III (11 patients) (%)* | *Group V (11 patients) (%)* |
| *Histology* | | | | | | | | | | |
| Squamous cell carcinoma | 97 | 98 | 97 | 95 | 95 | 98 | 100 | 100 | 91 | 100 |
| Adenocarcinoma | 2 | 2 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 |
| Undifferentiated carcinoma | 1 | 0 | 3 | 0 | 0 | 2 | 0 | 0 | 9 | 0 |
| *Substages* | | | | | | | | | | |
| IIa (vaginal extension) | 42 | 40 | 42 | 48 | 40 | - | - | - | - | - |
| IIb (parametrial extension) | 58 | 60 | 58 | 52 | 60 | - | - | - | - | - |
| *Age distribution (years)* | | | | | | | | | | |
| 27-29 | 3 | 4 | 7 | 0 | 0 | 4 | 0 | 10 | 0 | 9 |
| 30-39 | 21 | 13 | 30 | 42 | 5 | 13 | 27 | 10 | 0 | 9 |
| 40-49 | 29 | 31 | 30 | 24 | 30 | 23 | 13 | 50 | 9 | 27 |
| 50-59 | 20 | 21 | 30 | 5 | 15 | 36 | 27 | 20 | 64 | 37 |
| 60-69 | 18 | 23 | 3 | 24 | 25 | 13 | 27 | 10 | 9 | 0 |
| 70-79 | 7 | 6 | 0 | 5 | 20 | 11 | 6 | 0 | 18 | 18 |
| 80-87 | 2 | 2 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *Socioeconomic status* | | | | | | | | | | |
| Service patients | 80 | 81 | 82 | 90 | 65 | 90 | 87 | 90 | 82 | 100 |
| Private patients | 20 | 19 | 18 | 10 | 35 | 10 | 13 | 10 | 18 | 0 |

**Table III.** Results of therapy

| | *Stage II* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Total intact uteri (122 patients) (%)* | *Group I (48 patients) (%)* | *Group II (33 patients) (%)* | *Group III (21 patients) (%)* | *Group V (20 patients) (%)* | *Group VI (4 patients) (%)* | *Total patients (126 patients) (%)* |
| Living without cancer | 36.9 | 20.8 | 60.6 | 42.8 | 30.0 | 25.0 | 36.4 |
| Living with recurrence | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Died of cancer | 52.4 | 68.8 | 27.3 | 42.8 | 65.0 | 75.0 | 52.4 |
| Died of intercurrent disease | 7.4 | 10.4 | 9.1 | 0.0 | 5.0 | 0.0 | 7.4 |
| Lost to follow-up | 0.8 | 0.0 | 3.0 | 0.0 | 0.0 | 0.0 | 0.8 |
| Died of treatment without demonstrable cancer | 2.5 | 0.0 | 0.0 | 14.4 | 0.0 | 0.0 | 2.5 |
| Follow-up — Range | — | 5-15 years | 3-14 years | 22-55 months | 2-15 years | — | — |
| Median | — | 11.5 years | 7.5 years | 41 months | 12.5 years | — | — |

the survival of these patients in Stages I, II, and III are given in Table V. Nineteen instances of residual tumor may be described as follows: lymph node metastases, 10; radioresistant cancer as evidenced by local persistence in the cervix or adjacent vagina, 11; miscellaneous, 1. In 3 cases, node metastases and radioresistance were concurrent.

The incidence of cancer-bearing lymph nodes was 11 per cent, 18 per cent, and 10 per cent for Stages I, II, and III, respectively. This is well below that anticipated for each stage before treatment. Three patients with positive nodes are living after 5, 10, and 10 years, respectively. However, the generally reported low survival in such patients and our own experience with very late pelvic brim and aortic node recurrence strongly suggest that these patients can be considered only as representative of chance good fortune rather than success of the treatment method.

Eleven patients, or 12 per cent of the entire group, had radioresistant lesions. Of the 8 patients with local persistence alone, 7 are living without cancer 4, 7, 8, 8, 11, 13, and 13 years. These appear to represent treatment successes. One patient with tumor in the parametrial lymphatics died of pulmonary metastases without evidence of pelvic cancer.

The one patient with residual tumor in one Fallopian tube alone occupies an intermediate position but survival without cancer after 5 years is considered a treatment success. Over-all, 11 patients, or 58 per cent of those with residual cancer, appear to have been cured or effectively palliated by combined therapy.

### Statistical analysis

**Methods.** The composition of the standard radiation and the combined therapy groups of patients was tested within and between the groups for heterogeneity to all measured variables by the method of chi square.

Survival was analyzed by the actuarial or life table method as described by Cutler and Ederer.[3, 4] The method as utilized in this paper has the following advantages:

1. It makes possible the use of all survival information accumulated up to the closing date of the study.

2. By use of cumulative survival rates, results are interpreted as if all patients were treated on the same day.

3. It treats patients as if dead at the time recurrent tumor was noted since they represent primary treatment failures.

4. It assumes that subsequent to the date of last contact the survival experience of patients lost to follow-up is similar to that of patients remaining under observation (the possible fallacy of this assumption is minimized in this particular study since only one patient was lost to follow-up).

| Stage III | | | | | | |
|---|---|---|---|---|---|---|
| *Total intact uteri (47 patients) (%)* | *Group I (15 patients) (%)* | *Group II (10 patients) (%)* | *Group III (11 patients) (%)* | *Group V (11 patients) (%)* | *Group VI (3 patients) (%)* | *Total patients (50 patients) (%)* |
| 29.8 | 13.3 | 90.0 | 18.2 | 9.1 | 66.6 | 32.0 |
| 2.1 | 0.0 | 0.0 | 9.1 | 0.0 | 0.0 | 2.0 |
| 68.1 | 86.7 | 10.0 | 72.7 | 90.9 | 33.3 | 66.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| — | — | — | — | — | — | — |
| — | — | — | — | — | — | — |
| — | 6-9 years | 8-13 years | 24-32 months | 6 years | — | — |
| — | 7.5 years | 10 years | 27 months | 6 years | — | — |

5. It assumes that patients lost or withdrawn during each yearly interval were exposed to the risk of dying, on the average, for half of the interval.

6. Since each yearly interval studied contributed some information about patient survival, experience with patients observed for only a portion of the entire study time can be utilized. Therefore, computation of statistically valid 15 year survival rates is possible for the present groups where usual methods of survival calculation would permit only 5 year rates.

Standard errors for cumulative survival rates can be computed and indicate the extent to which the survival rates have been influenced by sampling error. From the cumulative survival rates and their respective standard errors, tests of the significance of differences in survival rates can be performed.

**Results.** Within the standard radiation and the combined therapy groups, patient composition with respect to age and socioeconomic status and stage, substage, and histology of the tumors was homogeneous (Table VI). Between groups, similar homogeneity was present except for age distribution. Differences in this variable were highly significant ($P < 0.001$) with more older and fewer young patients in the standard radiation group and the reverse in the combined therapy group. Fifteen-year cumulative survival rates between patients over and under 45 years of age in each group were then compared and no significant differences were found (Table VII). It was assumed therefore that comparisons between therapy groups would now be valid since the composition of each group was either homogeneous or if heterogeneous, as with age distribution, would not significantly alter the primary variable, survival.

Complete life tables for the standard radiation and combined therapy groups are given in Tables VIII and IX. The results of the life table analyses and the probability values for the differences in survival observed are listed in Table X. Cumulative survival rates have also been calculated after deletion of the first year's experience and are expressed as x-1 years. As noted by Arneson,[1] exclusion of this experience tends to minimize selection of patients in whom the initial prognosis was unfavorable by virtue of tumor virulence, tumor radioresistance, presence of unrecognized metastases, host resistance, or many other as yet unknown factors.

The difference between 16-1 year cumulative survival rates is highly significant ($P_{16\text{-}1} = 0.004$). If improved survival were due solely to removal of demonstrated residual tumor, deletion of patients with positive surgical pathologic conditions from the combined therapy group should eliminate the difference. Such tables were computed

and the results are given in Table X. However, rather than being nullified, the difference in survival remains essentially unchanged ($P_{16\text{-}1} = 0.002$).

In an effort to elucidate this finding, similar tables were constructed for each stage of cancer under consideration. The results are summarized in Table X and show that significantly improved survival with combined therapy was obtained in Stage II ($P_{16\text{-}1} = 0.004$) and Stage III ($P_{16\text{-}1} = 0.006$) but not in Stage I ($P_{16\text{-}1} > 0.20$).

### Comment

**The actuarial method.** Usually, survival rates after therapy for gynecologic cancer have been based on the absolute number of surviving patients and the actual duration of

**Table IV.** Severe complications of therapy

| | *Stage II* | | | | *Stage III* | | | |
|---|---|---|---|---|---|---|---|---|
| | *Group I (%)* | *Group II (%)* | *Group III (%)* | *Group III plus operation (%)* | *Group I (%)* | *Group II (%)* | *Group III (%)* | *Group III plus operation (%)* |
| Fistula | 0 | 9 | 0 | 29 | 0 | 20 | 0 | — |
| Bladder | 0 | 9 | 0 | 29 | 0 | 0 | 0 | — |
| Rectum | 0 | 0 | 0 | 0 | 0 | 20 | 0 | — |
| Proctitis 2°* | 2 | 3 | 10 | 14 | 0 | 10 | 9 | — |
| Cystitis 2°† | 0 | 0 | 10 | 0 | 0 | 0 | 9 | — |
| Excessive pelvic fibrosis | 0 | 0 | 14 | 29 | 0 | 0 | 0 | — |
| Pyometra | 2 | 0 | 0 | 0 | 7 | 0 | 0 | — |
| Paralysis, leg | 0 | 0 | 0 | 0 | 0 | 0 | 9 | — |
| Lignification | 0 | 0 | 5 | 14 | 0 | 0 | 27 | — |
| Lymphocele | 0 | 0 | 0 | 14 | 0 | 0 | 0 | — |
| Pelvic necrosis | 0 | 0 | 0 | 29 | 0 | 0 | 0 | — |
| Deaths | 2 | 0 | 0 | 43 | 0 | 0 | 9 | — |
| Total patients complicated | 6 | 12 | 29 | 72 | 7 | 30 | 46 | — |

*Proctitis 2° = radiation proctitis, ulcer, bleeding, or mild stenosis.

†Cystitis 2° = radiation cystitis, ulcer, or bleeding.

**Table V.** Incidence and location of residual tumor after combined therapy

| *Stage* | *Site* | *No. cases* | *Survival* |
|---|---|---|---|
| I (50 cases) (22 without pelvic lymphadenectomy) | Lymph nodes ± cervix | 3 (10.7%) | |
| | Nodes alone | 2 | Died 10 to 18 months with pelvic cancer |
| | Nodes and cervix | 1 } (8.0%)* | Died 11 months with pelvic cancer |
| | Cervix alone | 3 } | Living 4, 8, 13 years without cancer |
| | Total | 6 (12.0%) | |
| II (33 cases) | Lymph nodes ± cervix | 6 (18.2%) | |
| | Nodes alone | 4 | 3 living 5, 10, 10 years without cancer<br>1 died 21 months with pelvic cancer |
| | Nodes and cervix | 2 } | Died 13 and 18 months with pelvic cancer |
| | Cervix alone | 2 } (15.2%)* | Living 7 and 8 years without cancer |
| | Cervix and parametrial lymphatics | 1 } | Died 29 months with distant cancer only |
| | Fallopian tube | 1 | Living 5 years without cancer |
| | Total | 10 (30.3%) | |
| III (10 cases) | Lymph nodes alone | 1 (10.0%) | Died 32 months with pelvic cancer |
| | Cervix or upper vagina | 2 (20.0%)* | Living 11 and 13 years without cancer |
| | Total | 3 (30.0%) | |

*Incidence of radioresistant lesions.

Table VI. Analysis of group variables other than therapy

| | *Group I standard radiation* | | | | | | *Group II combined therapy* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Stage I (%)* | *Stage II (%)* | *Stage III (%)* | *All stages (%)* | *Intragroup heterogeneity* | *Intergroup heterogeneity* | *Intragroup heterogeneity* | *Stage I (%)* | *Stage II (%)* | *Stage III (%)* | *All stages (%)* |
| *Age (years)* | | | | | $P > 0.50$ | $P < 0.001$ | $P > 0.25$ | | | | |
| 24-29 | 0 | 4 | 0 | 2 | | | | 2 | 7 | 10 | 4 |
| 30-39 | 16 | 13 | 27 | 16 | | | | 50 | 30 | 10 | 39 |
| 40-49 | 34 | 31 | 13 | 30 | | | | 28 | 30 | 50 | 31 |
| 50-59 | 24 | 21 | 27 | 23 | | | | 20 | 30 | 20 | 24 |
| 60-69 | 21 | 23 | 27 | 22 | | | | 0 | 3 | 10 | 2 |
| 70-79 | 5 | 6 | 6 | 6 | | | | 0 | 0 | 0 | 0 |
| 80-89 | 0 | 2 | 0 | 1 | | | | 0 | 0 | 0 | 0 |
| *Histology* | | | | | $P > 0.50$ | $P > 0.05$ | $P > 0.20$ | | | | |
| Squamous cell carcinoma | 97 | 98 | 100 | 98 | | | | 90 | 97 | 100 | 94 |
| Adenocarcinoma | 3 | 2 | 0 | 2 | | | | 6 | 0 | 0 | 3 |
| Undifferentiated carcinoma | 0 | 0 | 0 | 0 | | | | 4 | 3 | 0 | 3 |
| *Socioeconomic status* | | | | | $P > 0.10$ | $P > 0.50$ | $P > 0.10$ | | | | |
| Service | 68 | 81 | 87 | 77 | | | | 68 | 82 | 90 | 75 |
| Private | 32 | 19 | 13 | 23 | | | | 32 | 18 | 10 | 25 |
| *Substage* | | | | | — | | — | | | | |
| Ia | 5 | — | — | — | | $P > 0.25$ | | 8 | — | — | — |
| Ib | 37 | — | — | — | | | | 24 | — | — | — |
| Ic | 58 | — | — | — | | | | 68 | — | — | — |
| IIa | — | 40 | — | — | | $P > 0.75$ | | — | 42 | — | — |
| IIb | — | 60 | — | — | | | | — | 58 | — | — |
| *Stage* | | | | | — | $P > 0.05$ | — | | | | |
| I (patients) | 38 | — | — | — | | | | 50 | — | — | — |
| II (patients) | — | 48 | — | — | | | | — | 33 | — | — |
| III (patients) | — | — | 15 | — | | | | — | — | 10 | — |

survival without demonstrable malignancy. With this method, one is often at a loss to know what to do with patients lost to follow-up or dead of intercurrent disease. If they were free of cancer at the time of last examination, they might be placed in the surviving without cancer group but it is obvious that this would prejudice the resultant survival rate since at least some of the patients were not free from cancer. Results would be similarly prejudiced if all of these patients were assumed dead of malignancy. Often, such patients are simply so categorized when presenting results. However, this has the effect of assuming them dead of cancer since the average observer notes only the surviving without cancer statistics. With so many different ways of stating the same thing, one is often at a loss to compare results from different series. The actuarial method used in this report treats such patients in a statistically valid manner neither completely including nor completely deleting them from the study. The method is valid if the life tables are constructed from rigorously controlled material.

It is becoming more and more obvious that 5 year survival rates are inadequate to interpret therapeutic regimes properly. Ten and 15 year survival rates broaden our perspective and may completely alter interpretation of results. The objection to such prolonged survival rates is the even longer time required to amass sufficient material and follow-up observations. The actuarial method permits calculation of long-term survival rates for patients having varying periods of follow-up observations. Thus, the

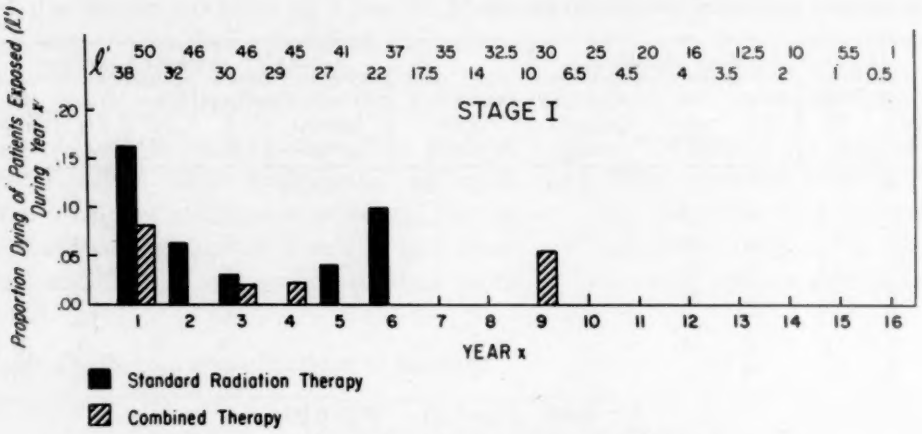


**Fig. 1.** Occurrence of deaths from cancer after therapy.

total time required to compute statistically valid long-term survival rates is significantly reduced. In this study, the longest patient survival and therefore the longest calculable survival rate was 16 years. However, the study includes patients observed for shorter time intervals down to 3 years. By non-actuarial methods, an additional 13 years would have been required before similar data could have been obtained.

**Interpretation of results.** Elective radical operation following radiation therapy for cervical cancer was initiated in an effort to reduce the incidence of early and late pelvic recurrence. Presumably, the operation would remove residual carcinoma. Operation was not withheld from patients in whom the radiation response was good, nor was preoperative demonstration of persistent cancer a prerequisite. The operation was elective. It was not offered to patients in whom the tumor progressed during therapy or whose general condition was deteriorating.

This study shows that improved survival was attained from combined therapy for Stage I, II, and III patients as a group but

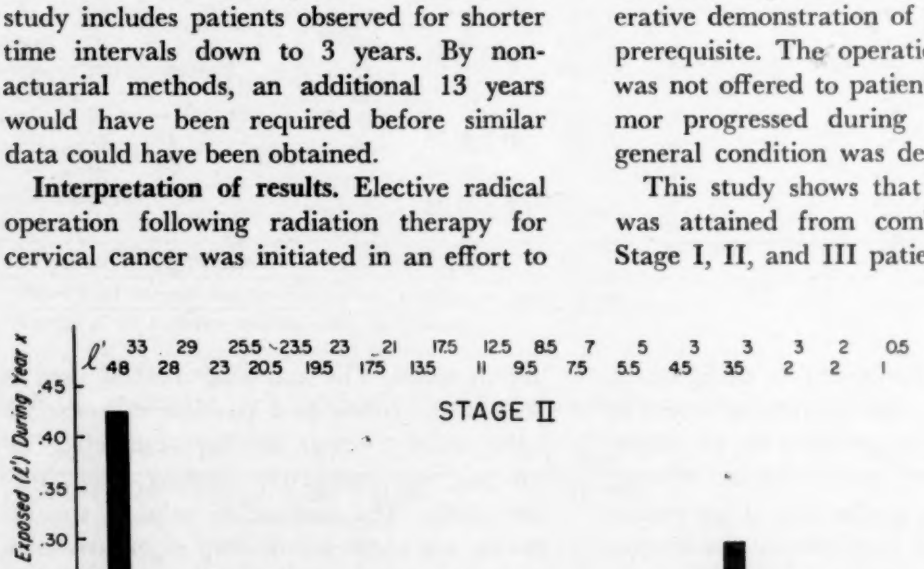


**Fig. 2.** Occurrence of deaths from cancer after therapy.

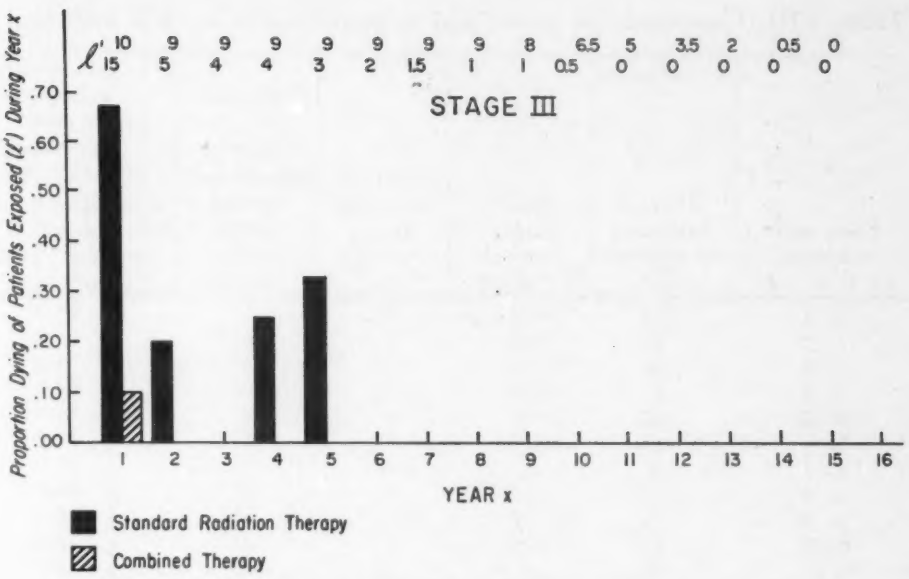


**Fig. 3.** Occurrence of deaths from cancer after therapy.

by a more subtle means. Cases where demonstrable residual cancer was removed do not explain the improved results. However, this fact does not alter the premise that small foci of carcinoma, cells destined to annihilate the host, were not removed. The premise is strengthened when survival rates are compared within each stage. Significantly improved survival was attained from combined therapy in Stages II and III where a high incidence of residual tumor would be anticipated. In Stage I, where the incidence of residual tumor should be low, survival was unaltered. Referring to Table X, we see that, after combined therapy, survival rates decrease only slightly between 5 and 10 years and then remain constant. However, in Stage II, after standard radiation alone, survival progressively decreases from 5 to 10 to 15 years and in larger gradations. Such late cancer deaths (Figs. 1-3) are infrequent in Stage I after either treatment method. These results further suggest that the incidence of residual cancer after radiation therapy for Stage I lesions is so low that additional elective surgical excision will not add significantly to survival.

The survival data from patients with positive surgical pathologic conditions suggest that undetected cancer cells have already spread distally by the time a site of demonstrable tumor has developed. When the tumor site is in the cervix or proximal parametrial tissues, radical resection of the uterus and pelvic lymph nodes is more apt to remove all undetected cancer (88 per cent survival), but if recognizable tumor is present at the periphery of the resection (lymph nodes) surgical treatment is probably inadequate (30 per cent survival). Therefore a negative surgical pathologic condition is prognostically good not because cancer cells are absent but rather because the chances of their successful removal are excellent. In this context, pelvic lymphadenectomy is an essential part of the radical operation since it adds so much to the scope of the resection

**Table VII.** Relationship of age and treatment method to survival (Stages I, II, and III)

| | *15 year cumulative survival* | |
|---|---|---|
| *Standard radiation* | | |
| 24-45 years | 0.28 ± 0.074 | $P > 0.50$ |
| 46 plus years | 0.36 ± 0.122 | |
| *Combined therapy* | | |
| 24-45 years | 0.74 ± 0.062 | $P > 0.05$ |
| 46 plus years | 0.88 ± 0.051 | |

**Table VIII.** Combined life tables and computation of survival rates and standard errors, standard

| *Years after diagnosis (x to x + 1)* | *Alive at beginning of interval ($l_x$)* | *Died during interval ($d_x$)* | *Lost to follow-up during interval ($u_x$)* | *Died of intercurrent disease during interval ($d.i.d_x$)* | *Withdrawn alive during interval ($w'_x$)* | *Effective number withdrawn alive during interval ($d.i.d_x + w'_x$) ($w_x$)* | *Effective number exposed to the risk of dying ($l_x - \frac{1}{2}u_x - \frac{1}{2}w_x$) ($l'_x$)* | *Proportion dying ($d_x/l'_x$) ($q_x$)* |
|---|---|---|---|---|---|---|---|---|
| 0- 1 | 101 | 37 | – | – | – | – | 101 | 0.37 |
| 1- 2 | 64 | 8 | – | – | – | – | 64 | 0.12 |
| 2- 3 | 56 | 3 | – | – | – | – | 56 | 0.05 |
| 3- 4 | 53 | 2 | – | 1 | – | 1 | 52.5 | 0.04 |
| 4- 5 | 50 | 1 | – | 2 | 4 | 6 | 47 | 0.02 |
| 5- 6 | 43 | 3 | – | – | 4 | 4 | 41 | 0.07 |
| 6- 7 | 36 | – | – | 2 | 5 | 7 | 32.5 | – |
| 7- 8 | 29 | – | – | – | 6 | 6 | 26 | – |
| 8- 9 | 23 | 1 | – | 1 | 4 | 5 | 20.5 | 0.05 |
| 9-10 | 17 | 1 | – | – | 4 | 4 | 15 | 0.07 |
| 10-11 | 12 | – | – | – | 2 | 2 | 11 | – |
| 11-12 | 10 | – | – | – | 1 | 1 | 9.5 | – |
| 12-13 | 9 | 1 | – | – | 3 | 3 | 7.5 | 0.13 |
| 13-14 | 5 | – | – | – | 2 | 2 | 4.0 | – |
| 14-15 | 3 | – | – | – | 1 | 1 | 2.5 | – |
| 15-16 | 2 | – | – | – | 2 | 2 | 1.0 | – |

**Table IX.** Combined life table and computation of survival rates and standard errors, combine

| *Years after diagnosis (x to x + 1)* | *Alive at beginning of interval ($l_x$)* | *Died during interval ($d_x$)* | *Lost to follow-up during interval ($u_x$)* | *Died of intercurrent disease during interval ($d.i.d_x$)* | *Withdrawn alive during interval ($w'_x$)* | *Effective number withdrawn alive during interval ($d.i.d_x + w'_x$) ($w_x$)* | *Effective number exposed to the risk of dying ($l_x - \frac{1}{2}u_x - \frac{1}{2}w_x$) ($l'_x$)* | *Proportion dying ($d_x/l'_x$) ($q_x$)* |
|---|---|---|---|---|---|---|---|---|
| 0- 1 | 93 | 8 | – | – | – | – | 93 | 0.09 |
| 1- 2 | 85 | 4 | – | – | – | – | 85 | 0.05 |
| 2- 3 | 81 | 2 | – | 1 | – | 1 | 80.5 | 0.03 |
| 3- 4 | 78 | 1 | – | – | 1 | 1 | 77.5 | 0.01 |
| 4- 5 | 76 | – | – | – | 6 | 6 | 73 | – |
| 5- 6 | 70 | – | – | – | 6 | 6 | 67 | – |
| 6- 7 | 64 | 1 | – | – | 6 | 6 | 61 | 0.02 |
| 7- 8 | 57 | – | – | 2 | 6 | 8 | 53 | – |
| 8- 9 | 49 | 1 | 1 | – | 5 | 5 | 46 | 0.02 |
| 9-10 | 42 | – | – | – | 8 | 8 | 38 | – |
| 10-11 | 34 | – | – | – | 12 | 12 | 28 | – |
| 11-12 | 22 | – | – | 1 | 3 | 4 | 20 | – |
| 12-13 | 18 | – | – | – | 4 | 4 | 16 | – |
| 13-14 | 14 | – | – | – | 5 | 5 | 11.5 | – |
| 14-15 | 9 | – | – | – | 4 | 4 | 7 | – |
| 15-16 | 5 | – | – | – | 5 | 5 | 2.5 | – |

adiation therapy (Stages I, II, and III)

| Proportion dying ($d_x/l'_x$) ($q_x$) | Proportion surviving ($1 - q_x$) ($p_x$) | Cumulative proportion surviving from diagnosis through end of interval ($p_1 Xp_2X \ldots Xp_x$) ($P_x$) | Cumulative proportion surviving from year 1 to end of interval ($p_2 Xp_3X \ldots Xp_x$) ($P_{x-1}$) | $l'_x - d_x$ | $q_x/l'_x - d_x$ | Standard error $S.E._x = P_x \sqrt{\sum_{x=1}^{x} \frac{q_x}{l'_x - d_x}}$ | |
|---|---|---|---|---|---|---|---|
| 0.37 | 0.63 | 0.63 | – | 64 | 0.0057 | | |
| 0.12 | 0.88 | 0.56 | 0.88 | 56 | 0.0022 | | |
| 0.05 | 0.95 | 0.52 | 0.84 | 53 | 0.0010 | | |
| 0.04 | 0.96 | 0.50 | 0.80 | 50.5 | 0.0008 | | |
| 0.02 | 0.98 | 0.49 | 0.79 | 46 | 0.0005 | | ±0.049 |
| 0.07 | 0.93 | 0.46 | 0.73 | 38 | 0.0019 | | |
| – | 1.00 | 0.46 | 0.73 | 32.5 | – | | |
| – | 1.00 | 0.46 | 0.73 | 26 | – | | |
| 0.05 | 0.95 | 0.44 | 0.70 | 19.5 | 0.0025 | | |
| 0.07 | 0.93 | 0.41 | 0.65 | 14 | 0.0048 | | ±0.057 |
| – | 1.00 | 0.41 | 0.65 | 11 | – | | |
| – | 1.00 | 0.41 | 0.65 | 9.5 | – | | |
| 0.13 | 0.87 | 0.35 | 0.56 | 6.5 | 0.0204 | | |
| – | 1.00 | 0.35 | 0.56 | 4 | – | | |
| – | 1.00 | 0.35 | 0.56 | 2.5 | – | | ±0.05 |
| – | 1.00 | 0.35 | 0.56 | 1 | – | (16 – 1) | ±0.105 |

herapy (Stages I, II, and III)

| Proportion dying ($d_x/l'_x$) ($q_x$) | Proportion surviving ($1 - q_x$) ($p_x$) | Cumulative proportion surviving from diagnosis through end of interval ($p_1 Xp_2X \ldots Xp_x$) ($P_x$) | Cumulative proportion surviving from year 1 to end of interval ($p_2 Xp_3X \ldots Xp_x$) ($P_{x-1}$) | $l'_x - d_x$ | $q_x/l'_x - d_x$ | Standard error $S.E._x = P_x \sqrt{\sum_{x=1}^{x} \frac{q_x}{l'_x - d_x}}$ | |
|---|---|---|---|---|---|---|---|
| 0.09 | 0.91 | 0.91 | – | 85 | 0.0010 | | |
| 0.05 | 0.95 | 0.86 | 0.95 | 81 | 0.0006 | | |
| 0.03 | 0.97 | 0.83 | 0.93 | 78.5 | 0.0003 | | |
| 0.01 | 0.99 | 0.82 | 0.92 | 76.5 | 0.0001 | | |
| – | 1.00 | 0.82 | 0.92 | 73 | – | | ±0.037 |
| – | 1.00 | 0.82 | 0.92 | 67 | – | | |
| 0.02 | 0.98 | 0.80 | 0.90 | 60 | 0.0003 | | |
| – | 1.00 | 0.80 | 0.90 | 53 | – | | |
| 0.02 | 0.98 | 0.78 | 0.88 | 45 | 0.0004 | | |
| – | 1.00 | 0.78 | 0.88 | 38 | – | | ±0.041 |
| – | 1.00 | 0.78 | 0.88 | 28 | – | | |
| – | 1.00 | 0.78 | 0.88 | 20 | – | | |
| – | 1.00 | 0.78 | 0.88 | 16 | – | | |
| – | 1.00 | 0.78 | 0.88 | 11.5 | – | | |
| – | 1.00 | 0.78 | 0.88 | 7 | – | | ±0.041 |
| – | 1.00 | 0.78 | 0.88 | 2.5 | – | (16-1) | ±0.036 |

**Table X.** Cumulative survival according to method of treatment

| | *Years at risk* | *Standard radiation* | *Combined therapy* | *Combined therapy (patients with negative surgical pathology only)* |
|---|---|---|---|---|
| Combined Stages I, II, and III | 5 | 0.49 ± 0.049 | 0.82 ± 0.039 | 0.90 ± 0.034 |
| | 10 | 0.41 ± 0.057 | 0.78 ± 0.043 | 0.87 ± 0.042 |
| | 15 | 0.35 ± 0.050 | 0.78 ± 0.043 | 0.87 ± 0.042 |
| | 16-1* | 0.56 ± 0.105 | 0.88 ± 0.036 | 0.90 ± 0.037 |
| | | | ($P_{16-1} = 0.004$)‡ | ($P_{16-1} = 0.002$)‡ |
| Stage I | 5 | 0.74 ± 0.072 | 0.88 ± 0.044 | 0.94 ± 0.038 |
| | 10 | 0.70 ± 0.076 | 0.85 ± 0.051 | 0.90 ± 0.050 |
| | 15 | 0.70 ± 0.076 | 0.85 ± 0.051 | 0.90 ± 0.050 |
| | 16-1* | 0.83 ± 0.069 | 0.93 ± 0.037 | 0.94 ± 0.041 |
| | | | ($P_{16-1} > 0.20$)‡ | ($P_{16-1} > 0.10$)‡ |
| Stage II | 5 | 0.41 ± 0.071 | 0.76 ± 0.074 | 0.82 ± 0.080 |
| | 10 | 0.28 ± 0.074 | 0.71 ± 0.082 | 0.76 ± 0.093 |
| | 15 | 0.20 ± 0.086 | 0.71 ± 0.082 | 0.76 ± 0.093 |
| | 16-1* | 0.35 ± 0.144 | 0.81 ± 0.077 | 0.84 ± 0.087 |
| | | | ($P_{16-1} = 0.004$)‡ | ($P_{16-1} = 0.003$)‡ |
| Stage III | 5 | 0.13 ± 0.085 | 0.90 ± 0.095 | 1.00 ± 0.00 |
| | 10 | 0.13 ± 0.085 | 0.90 ± 0.095 | 1.00 ± 0.00 |
| | 11-1† | 0.40 ± 0.219 | 1.00 ± 0.00 | 1.00 ± 0.00 |
| | | | ($P_{16-1} = 0.006$)‡ | ($P_{16-1} = 0.006$)‡ |

*Indicates 15 year cumulative survival after deletion of first year experience.

†Indicates 10 year cumulative survival after deletion of first year experience.

‡P values refer to comparison with standard radiation group.

**Table XI.** Severe complications of therapy

| | *Stage I (%)* | *Stage II (%)* | *Stage III (%)* | *All stages (%)* |
|---|---|---|---|---|
| *Standard radiation* | | | | |
| Pulmonary embolus | 3 | 0 | 0 | 1 |
| Pyometra | 0 | 2 | 7 | 2 |
| Proctitis 2°* | 0 | 2 | 0 | 1 |
| Death (intestinal necrosis) | 0 | 2 | 0 | 1 |
| Total | 3 | 6 | 7 | 5 |
| *Combined therapy* | | | | |
| Fistula | 6 | 9 | 20 | 9 |
| Vesico- or urethrovaginal | 4 | 9 | 0 | 6 |
| Rectovaginal or vesical | 2 | 0 | 20 | 3 |
| Proctitis | 8 | 3 | 10 | 6 |
| 2°* | 6 | 3 | 10 | 5 |
| 3°* | 2 | 0 | 0 | 1 |
| Ureteral stricture | 2 | 0 | 0 | 1 |
| Pelvic abscess | 2 | 0 | 0 | 1 |
| Pulmonary embolus | 4 | 0 | 0 | 2 |
| Death, operative | 2 | 0 | 0 | 1 |
| Total | 24 | 12 | 30 | 20 |
| Surgically induced | 16 | 9 | 20 | 14 |
| Radiation induced | 8 | 3 | 10 | 6 |

*Proctitis 2° = radiation proctitis, ulcer, bleeding or mild stenosis. Proctitis 3° = radiation stricture requiring colostomy.

by removing sites of cancer spread as distal from the cervix as possible.

**Complications of therapy.** A final interpretation of the data cannot be made without consideration of the complication rates attendant to radical operation. Combined therapy increased severe complications threefold over those after standard radiation alone (Table XI). Does the improved survival justify the means?

There is little doubt that patients with Stage I lesions suffered more complications without statistically significant improved survival. For these, supplemental elective operation after standard radiation has no place. However, if demonstrable cancer persists after standard radiation therapy, the complications attendant to radical operation become only a calculated risk.

Patients in Stages II and III had statistically significant improved survival rates commensurate with the increased number of complications. Moreover, with the exception of deaths, tissues remained sufficiently normal to permit corrective surgical procedures

or spontaneous resolution of the complications in every case. Thus, essentially normal-functioning cancer-free patients ultimately were obtained.

## Conclusions

1. The actuarial method of survival analysis after cancer therapy provides maximum information about all patients observed during a study period.

2. There is no statistically significant difference in the survival of patients with Stage I cervical cancer treated with standard radiation therapy alone and those treated with supplemental elective radical operations ($P_{16\text{-}1} > 0.20$). After operation, severe complications increased threefold and contraindicated the treatment method.

3. The survival rate of patients with Stage II or Stage III cervical cancer is improved after combined radiation and elective radical operation. Statistically, the difference is highly significant ($P_{16\text{-}1} = 0.004$ and 0.006). Increased therapy complications are compatible with the improved survival.

4. Removal of residual cancer undetected by our tissue study methods probably explains improved survival after combined therapy.

We wish to acknowledge the advice and guidance of Dr. Harold O. Goodman, Department of Medical Genetics, Bowman Gray School of Medicine of Wake Forest College, during the preparation and evaluation of the statistical data.

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## Discussion

Dr. W. Norman Thornton, Jr., Charlottesville, Virginia. There are certain aspects of this presentation that need additional clarification. I wonder if the patients in the various classifications are homogeneous, since the dosage delivered to the cervix by standard and intensive therapy in the two groups is essentially the same. In addition to this fact, there is a wide variation, as much as 50 to 100 per cent, in the amount of irradiation delivered to point A by the various modalities. In evaluating the results, in small groups of patients, these factors are of significance and speak against the homogeneity of a particular group. In addition to this obvious variable, there is always the problem of variations in the extent of tumor in a particular stage and the classification of the clinical extent of the tumor even though all patients were classified by the same experienced gynecologist.

One wonders if great significance should be attached to the results obtained in applying life tables to small groups of patients in which there are variables as mentioned. In projecting end results in the treatment of cervical cancer one should also take into consideration the effects of operation, irradiation, and renal complications in addition to the malignancy, per se.

Finally, there is a disturbing aspect presented which should be considered. This paper has suggested and revived a concept which is difficult to accept. A concept which implies that preoperative irradiation in some inoperable Stage II and Stage III lesions permits resection by radical hysterectomy and pelvic lymphadenectomy.

The treatment of advanced cervical cancer is discouraging, and as shown in this series, is only palliative for many patients regardless of the type of therapy used and employing all methods available to us. Our best weapon is to promote the wider utilization of cytologic screening meth-

ods available to us in the early detection of curable cervical malignancy.

Dr. Axel N. Arneson, St. Louis, Missouri. I commend Dr. Lock for the use of life tables in the assessment of his therapeutic experiment. Cumulative data project long-term values by imposing upon all subsequent time intervals the weight of more immediate events. The later results are determined by the rate of dying from cancer, and for similar series of patients with the same variety of lesion. In the case of cervical cancer, it is largely determined during the first 5 years of observation.

With the opportunity to preview Dr. Lock's paper, I have plotted the rate of dying from cancer in the two series he compares. As you may have interpreted, the curve for the standard radiation group rises more steeply, approaching a plateau at the third and fourth year with its maximum attained at approximately the sixth year. The curve for combined therapy group approaches a plateau at the second and third years, and attains its maximum at approximately the fourth year. The latter curve corresponds more closely with favorable cases and with those often dying from disseminated disease.

One of our confreres in radiation therepy has wisely stated that more than half the weight of all factors affecting prognosis are predetermined by the stage of tumor's extension, physical qualities of the patient, and the unassessable nature of the tumor itself. Of importance but of lesser total significance is the skill and experience of the individual responsible for clinical management. The modality of treatment becomes the least significant factor, but it is the one over which we have greatest control.

Dr. Lock has tested his material for homogeneity in all assessable qualities of prognosis and finds comparability in every respect except for a moderate deviation in age. I am uncertain how he selects patients for elective operation but, mindful of the subconscious bias to which we are all subject and of the difficulties involved in assessing at the outset the true nature of the tumor, I would like to ask if he believes the statistically significant improvement in results is fully explained on the basis of differences in treatment alone.

Dr. Howard Ulfelder, Boston, Massachusetts. The question of selection of patients for radiation or for operation is tremendously influenced by many factors which are not measurable in a way that permits presentation in a table. This tendency to select is particularly true in the case of cancer of the cervix because there is available a form of therapy that is nonsurgical and, therefore, initially much less hazardous. It stands to reason that there will be selection when two forms of treatment are available, each of which has great possibilities for cure but one is risky and the other is much less risky at the time.

For these reasons I suggest comparisons no longer be made between groups of cases, some treated by one method and some by another. Comparisons in the future should be with all cases of the same stage. I do this with my own material. I am not interested whether the patients with a certain stage of cancer treated by radiation are doing as well as those treated by operation. I am looking to see whether all my Stage III patients, for example, are doing better than they used to do or how my results in this stage compare with those of other people.

Dr. Lock (Closing). Table VIII gives you some idea of the work involved in the preparation of 15 year cumulative life tables. This table is one of 26 which had to be done for all the patients in this series.

I was aware of the variation in irradiation dosage in this series. Rigid applicators were used in our plan of irradiation and individual isodose curves were made for each patient.

Dr. Arneson has asked if we are convinced that the addition of operative treatment is responsible for the significant difference in survival in this series of patients. The last patient in the Stage III group was subjected to elective surgical combined therapy 8 years ago. The last patient in the Stage II group was subjected to elective combined therapy 4 years ago. The reason for this lapse was our concern with the high incidence of complications, and our uncertainty as to whether we were obtaining improved results. From this analysis, I am convinced that we should resume this plan of treatment for Stage II and III carcinoma of the cervix, so as to permit further evaluation.

# Some factors affecting the fetal heart rate

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SINCE the fetal heart was first heard in the middle of the last century[1] there have been innumerable papers dealing with its variations and their prognostic significance as far as the life and well-being of the infant was concerned. Most of these papers deal with variations of the fetal heart rate in labor and emphasize the importance of slowing, particularly as it is associated with other signs of fetal distress. Since the advent of electronic reproduction, there have been numerous attempts at recording the fetal heart rate using both the phonocardiograph and the electrocardiograph.[2-3] These studies have been directed toward the diagnosis of fetal life and more recently toward continuous monitoring machines which would give continuous supervision of the fetal heart during parturition and serve as an automatic warning system at the first sign of fetal distress. While all of these projects are of considerable interest electronically, taken as a whole they have not yielded the significant clinical information that one could hope for. Furthermore, telemetering techniques in general have not yet made very significant strides in any field of medicine.

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In view of the vast amount of work already done in this field, one approaches further research on the fetal heart with the conviction that little significant new information can be garnered without the development of much more imaginative and sophisticated techniques than have hitherto been employed. More important, the objectives of most previous research have been diffuse and the questions asked have neither been sharply focused nor precisely answerable. However, inasmuch as the fetal heart furnishes the sole available contact with the infant in the intact human being, our studies were undertaken with the hope that if more advanced techniques could be employed and if specific answerable questions could be asked, interesting data might be obtained.

There are two possible areas of study: (1) the behavior of the fetal heart during the last half of pregnancy, and (2) the behavior of the fetal heart during labor. Of these two, we have chosen to study the former because the clinical problems involved are more simply defined, for example, the effect of serious maternal diseases such as toxemia or diabetes. In addition, the situation prior to labor lends itself to more easily controlled experimentation than does the time of labor and, finally, the specific question was asked: Can one find predictable differences in the response of the fetal heart to stimuli in well and nonwell fetuses? This naturally placed the time of investigation in the last trimester and not during parturition.

There are two possible methods of recording the fetal heart: the phonocardiograph

and the electrocardiograph. We have chosen the former because it seemed simpler for our purposes. With the latter there is always the problem of obliteration of the maternal complex which Hon and Hess[4] have apparently solved and, more difficult, the problem of obtaining sufficient energy from the fetal electrocardiograph to operate a rate meter without having a direct fetal lead. This is not to say that the phonocardiograph is without its own particular difficulties.

### The phonocardiograph

Our preliminary investigations were concerned with the characteristics of fetal heart sounds. On the phonocardiograph, each individual heart beat of the fetus is represented by two distinct sounds distributed in time. For example, at 120 beats per minute, the distribution of the two centroids occupies about 30 per cent of the cycle. The second sound is usually less loud than the first and its duration about one third of the 150 milliseconds required for the first sound. Even for a given individual fetal heart, the sounds have a wide variation in range of frequencies including some sound power at 5 cycles per second and ranging up to 100 cycles per second. In order to avoid interference from nonfetal sounds, a limited bandpass of frequencies has been used in our investigations. According to these preliminary studies, the maximum usable information is found at about 45 cycles per second, a region at the border line of ordinary human hearing.

Having obtained the above information, the problem was to develop a machine which could give a satisfactory recording of the beat-to-beat or instantaneous rate over a long period of time. Our first attempt was to use a sensitive microphone with a broad frequency capacity. A series of amplifiers, a bandpass filter, and an instantaneous rate meter, which was actually a modification of the Waters cardiotachometer, were used. With our first machine, we were able to show something of the beat-to-beat variations which could be expected from the normal fetal heart.[5] However, this apparatus had considerable difficulty with interference. Furthermore, there are variations in energy of the fetal heart sound from beat-to-beat which makes it almost impossible to use amplitude discrimination to operate the cardiotachometer (Fig. 1). All in all, this machine proved unsatisfactory for the recording of the long duration experiments with the fetal heart which we had in mind. After a year or two of rather discouraging work, an entirely new design was applied to the apparatus.[6] Borrowing the method of "track while scan" from radar systems, a unit was built which could track either the first or second fetal sound. In this system, the energy to operate the rate meter is derived from the center of a gate rather than from the fetal sound (Fig. 2). Once the machine is tracking, the geometric centroid of the fetal heart sound is centered within a gate and a gate is used to check the coincidence and detect any error of the centroid of the sound being tracked. The error signal, if any, modifies a gate prediction memory so as to cause


**Fig. 1.** Varying heights on this graphic record of fetal first and second heart sounds show the futility of amplitude discrimination as a means of operating a heart rate meter.

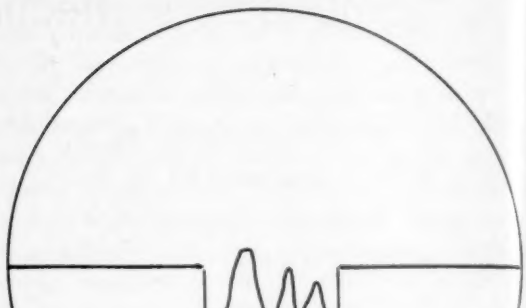

**Fig. 2.** Fetal heart sound centered in gate.

the gate in the next cycle to occur when the next burst of selected sound is expected. This time sampling gate, for error signal, reduces the interference from extraneous noise during the cycle period. The sweep repetition rate is adjusted during this gate but with a proportional error signal and longer memory to agree with the fetal heart rate.

To enable the operator to see that he has instituted accurate tracking, an oscilloscopic display of the sweep, gate, and sound is provided. This permits the operator to synchronize and phase manually the sweep and gate with the sound he desires to track and informs him when on automatic tracking of any loss of synchronization.

The display also helps the operator to select the exact bandpass suited to the individual fetal heart sound and coincidental noise. In addition, from the display, he can adjust the sound level.

This device has certain errors and limitations of recording as defined in Fig. 3, which is a graph that delineates the range of heart rate deviations that the cardiotachometer can properly measure. The graph is made in terms of the deviation of the heart rate ($\Delta R$) and the time increment ($\Delta T$) over which the deviation occurs. Those combinations of $\Delta R$ and $\Delta T$ that plot in the cross-hatched area of the graph are not correctly measured by the cardiotachometer; those that plot in the non–cross-hatched area are. The cross-hatched areas are caused by several factors. The top area is the result of failure of the system to track sudden large changes in rate. The area at the left is the result of the lowpass filter. The area at the lowest left (denoted signal artifact) is the result of small fluctuations in the energy distribution of the signal. The data recorded by this machine covering the normal range of maternal and fetal heart rates has an error of less than 1 per cent.

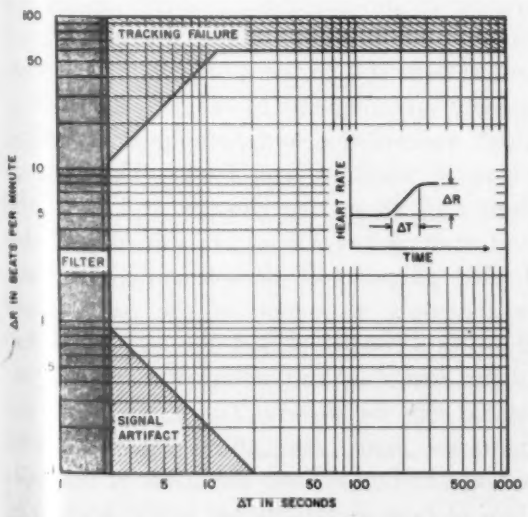


Fig. 3. Chart showing areas of tracking failure and signal error in relation to rate of change in the fetal heart beat.

Early in the investigation it became apparent that simple analysis of the rate recorded on paper tape could yield only the grossest kind of information. Even in the simple experiments to be described, the tapes run over an hour and the computing by hand of various means and difference of the instantaneous rate in response to stimuli is almost an insurmountable task. When one wishes to correlate changes in fetal rate with changes in maternal rate, the situation becomes even more difficult and complex. It is obvious that only a small portion of the information from any recording can be derived from simple inspection and, as will be shown presently, even this information can give exact numerical data only with difficulty and with the consumption of a large amount of time. It is therefore apparent that some sort of computor analysis of the information is necessary before quantitative conclusions can be derived from such experimentation. Accordingly, not only have the instantaneous fetal and maternal rates been recorded on paper tapes but the actual heart signatures are permanently recorded on magnetic tape, the maternal rate being recorded as the electrocardiogram and the fetal rate as the phonocardiogram. An analogue to digital converter has been designed. The magnetic tapes can be sent through a rate meter, the data being processed after having been converted to rate. The analogue to digital converter translates rate into voltage, sampling the analogue data at prescribed intervals. The information appears as coded paper tapes which can then be fed

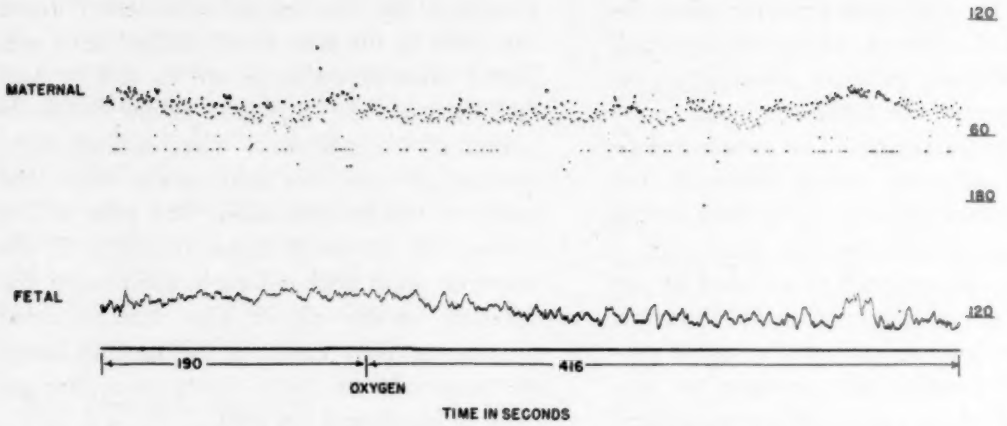


Fig. 4. The effect of 100 per cent oxygen on fetal and maternal heart rates.

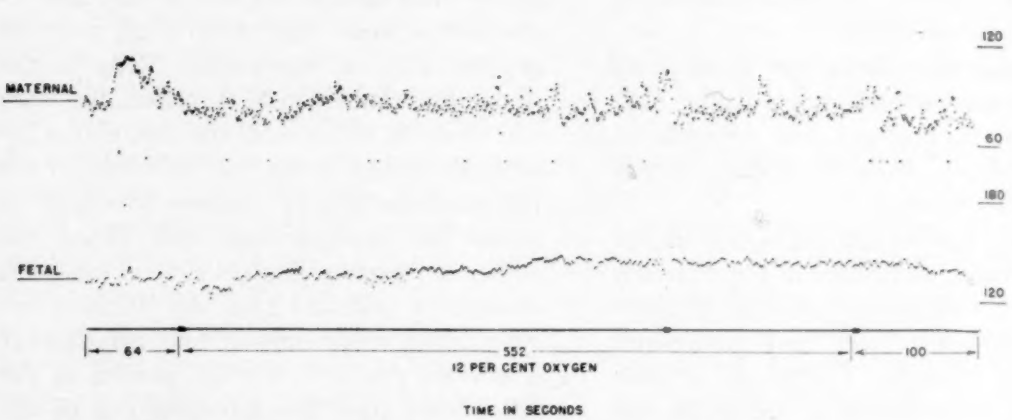


Fig. 5. The effect of 12 per cent oxygen on fetal and maternal heart rates.

into a computor and various parameters thereby numerically defined. The automatic data handling was not far enough developed to be utilized for this presentation. The data about to be presented were derived manually or semiautomatically.

**Observations with phonocardiograph of effect of stimuli to mother.** The question propounded in the present essay is preliminary to answering the major question referred to above. In this study, we have attempted to define the response of the normal fetal heart in the last trimester of normal pregnancy to a series of stimuli administered to the mother. The experiments all have a standard design, namely, a control period of recording of about 30 minutes, a period of administration of the stimulus, and a period of runout during which the effects of the stimulus and their gradual disappearance are observed. This period, including the time required for the administration of the stimulus, is usually about one hour. The following stimuli have been administered: 100 per cent oxygen, 12 per cent oxygen (equivalent to 13,000 feet elevation), epinephrine, norepinephrine, atropine, cigarette smoking, and fright. The over-all design of these experiments and the fetal and maternal responses are shown in Table I. For purposes of demonstration of these results, only a portion of the paper tapes showing a control period, the administration of the stimulus, and point of maximum response can be shown. The tapes show the maternal heart rate above and the fetal heart rate below. The duration of the portions of tape shown appear at the bottom of the slides with the time in seconds. A heavy mark appears on the time mark when syn-

chronization is lost. If portions of the tape are omitted, the length of time of omission is recorded but the blank areas are not necessarily proportional to marked areas.

*Exposure to 100 per cent oxygen.* In the oxygen experiments, a BLB mask was placed over the patient's nose and mouth. During the control period, she breathed air and then at the proper time either 100 per cent or 12 per cent oxygen was turned on. The experiment was fully explained to the patient beforehand and several practice periods using the mask both with and without oxygen were performed prior to the initiation of the experimental period. Fig. 4 shows the exposure of a mother to 100 per cent oxygen. In this particular figure, there is no maternal response to this stimulus; however, in others there was some flattening of the beat-to-beat variation and some slight slowing of the maternal pulse. The fetal response here is clear, showing an increase in the variability of the rate and a decrease in the rate. All in all, however, in the experiments done with 100 per cent oxygen, the changes were unpredictable.

*Exposure to 12 per cent oxygen.* Fig. 5 shows an experiment with 12 per cent oxygen. The duration of the stimulus in these experiments was reduced from 20 minutes with 100 per cent oxygen to 10 minutes because the mothers were uncomfortable breathing oxygen at this concentration for a more prolonged period. Again the maternal response is not marked here, although some mothers responded with a tachycardia and

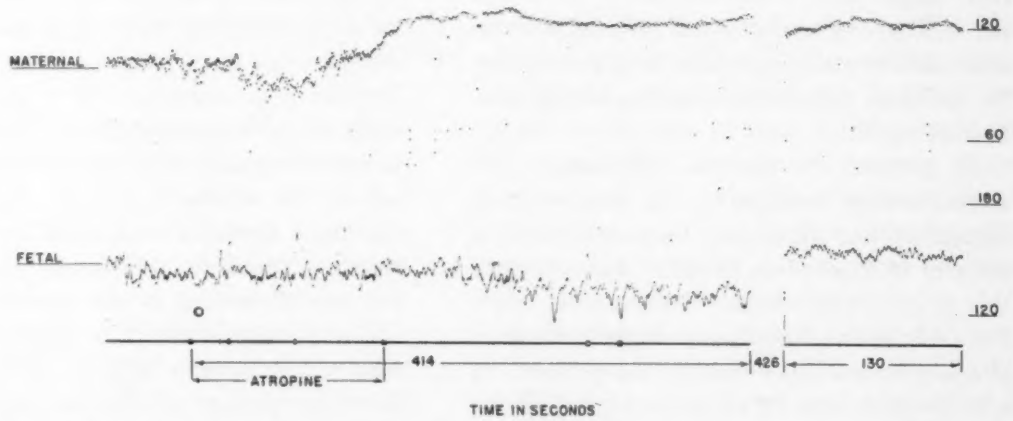


Fig. 6. The effect of atropine on fetal and maternal heart rates.

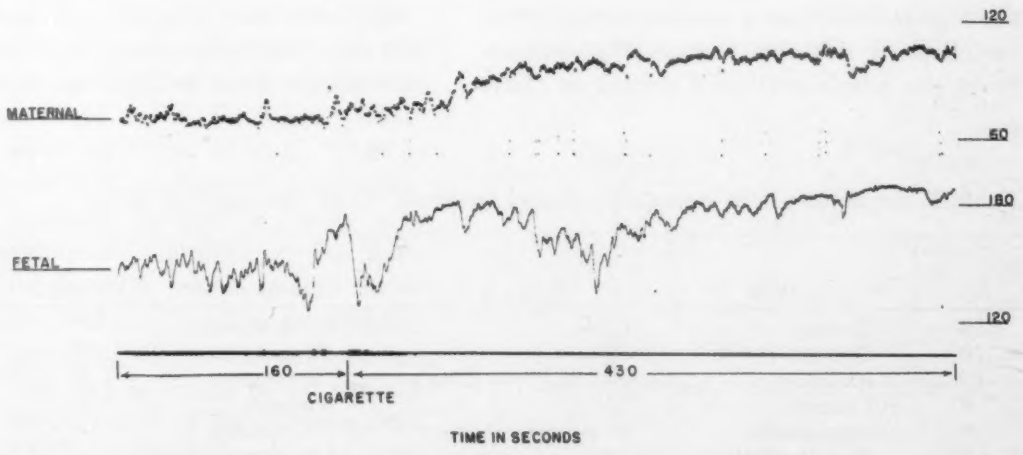


Fig. 7. The effect of cigarette smoking on fetal and maternal heart rates.

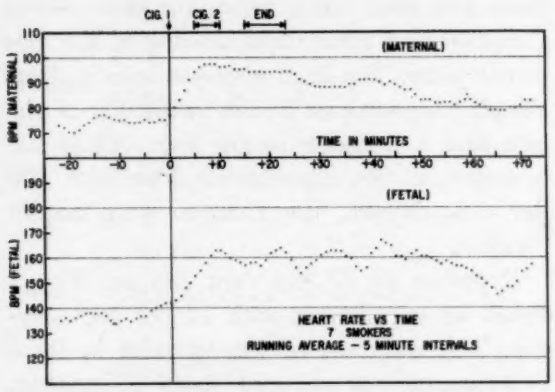


**Fig. 8.** Graph showing running mean of the effect of cigarette smoking on the fetal and maternal heart rates of 7 gravidas. Two cigarettes were used as stimuli.

a general flattening of the beat-to-beat variation. On the other hand, the fetal response is quite clear-cut, resulting in an increase in fetal heart rate of approximately 20 beats per minute beginning rather gradually sometime after the administration of the stimulus. In addition, there is a flattening of the beat-to-beat variation.

In general, the oxygen experiments were unsatisfactory because of the psychological impact of the mask. We have tried with a number of masks but all attempts to remove this psychological factor have proved fruitless. All masks have some slight resistance which subconsciously worries the patient. As will be seen later, this psychological factor has such a profound effect on the performance of the fetal heart that it becomes extremely difficult to interpret results of the above experiments in a valid statistical manner. Even if this were not so, the response to oxygen administration is neither as clear-cut nor as marked as will be seen with other stimuli.

*Administration of atropine.* Hon[7] was the first to point out to us that administration of atropine to the mother produced fetal tachycardia. This appeared to be a direct drug effect on the fetus and not a change in the maternal cardiac output or the uterine blood flow. If this were so then the type of fetal response and the time required to build up fetal vagal blockade should give some indication of the state of placental transfer. Such a response could be studied in relation to the time required for the maternal response which is particularly clear-cut.

Thirteen experiments were performed with atropine (Table II). After the usual control period with an intravenous solution of 5 per cent dextrose running, 1 mg. of atropine was given into the intravenous tube in a 2 minute period. The patients' conditions were then followed for 1 hour. Fig. 6 shows a typical experiment. Following the onset of the administration of atropine there is initial vagal stimulation producing a slowing in the mother's cardiac rate. As vagal blockade becomes established, the mother develops marked tachycardia with a pronounced flattening of the beat-to-beat variation. The same effect is visible in the fetal tracing but there is a time delay in order to allow for passage of the drug and sufficient build-up in the fetus to first produce vagal stimulation and then blockade.

The appearance times of maternal vagal stimulation and blockade are shown in Table II. It will be noted that slowing does not always occur in either the mother or the

**Table I.** Experiments in normal fetal and maternal heart response to stimuli

| *No.* | *Stimulus* | *Strength* | *Duration* | *Response* | |
|---|---|---|---|---|---|
| | | | | *Maternal* | *Fetal* |
| 10 | Oxygen | 100% | 20 minutes | ± | ± |
| 10 | Oxygen | 12% | 10 minutes | ± | + |
| 13 | Atropine | 0.5 mg. per minute | 2 minutes | + | + |
| 9 | Cigarette | 2 | Variable | + | + |
| 3 | Epinephrine | 20 μg per minute | 3 minutes | + | − |
| 3 | Norepinephrine | 4 μg per minute | 7 minutes | + | − |
| 1 | Fright | — | Variable | + | + |

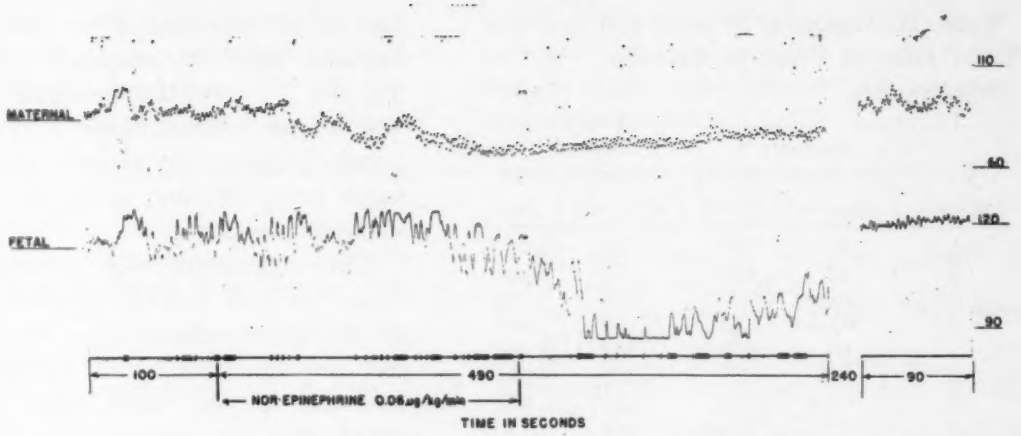


**Fig. 9.** The effect of norepinephrine on fetal and maternal heart rates.

fetus. On the other hand, with the dosage employed, vagal blockade always appears in the mother, although it did not occur in 2 of the 13 fetuses. The mean differences in time of occurrence of the two effects are shown. These are quite long, being in the neighborhood of 12 minutes for complete vagal blockade.

*Cigarette smoking.* Quite a different order of time delay is seen when cigarette smoking is used as a stimulus. Doerfel[8] and Sontag and Wallace[9] have demonstrated that cigarette smoking causes fetal tachycardia. They thought that this was a response to the passage of nicotine across the placenta. The time delays in our experiment are so short as to open this conclusion to question. In all, 10 experiments have been performed as shown in Table III. All patients were known habitual smokers. They were asked not to smoke for 24 hours prior to the test. Fig. 7 demonstrates a typical experiment with the usual control period. One or two cigarettes furnish the stimulus and the usual runout period of about one hour is used. There is a gradually increasing maternal tachycardia. Fetal tachycardia is usually quite pronounced with some flattening of the beat-to-beat variation. It occurs much more quickly than does the response to atropine, sometime preceding the maternal response. Not only this, but in 5 out of 10 patients there are short bursts of fetal tachycardia during the time when the mother is being given the cigarette but prior to its lighting. This type of response we have termed "anticipatory." The means for successive one minute periods in 7 patients have been determined semiautomatically and are presented as running means for 10 five minute periods in Fig. 8. This figure as well as the means shown in Table III demonstrate quite clearly the great rapidity of the fetal response in relation to that of the mother. It is this fact plus the frequent presence of the fetal anticipatory response that leads us to believe that fetal tachycardia with cigarette smoking is a response to some kind of vasomotor change in the placental bed.

*Epinephrine and norepinephrine.* Three patients were given epinephrine at the rate of 0.24 µg per kilogram per minute for 3 minutes. Although there was a rise in the maternal blood pressure, there was no change in the maternal or fetal heart rate. Similarly, 3 patients were given norepinephrine intravenously at the rate of 0.06 µg per

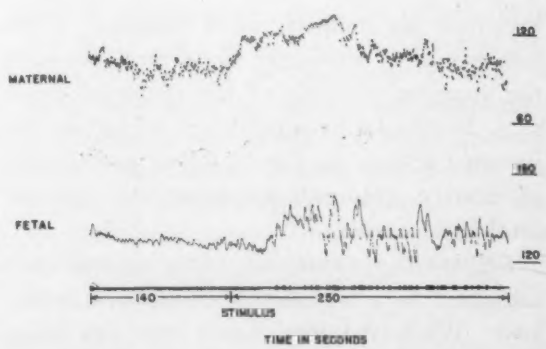


**Fig. 10.** The effect of fright on the maternal and fetal heart rates.

**Table II.** Response of fetal and maternal heart rates to 1 mg. of atropine intravenously

| Case | Seconds to maximum slowing | | Seconds to vagal blockade | |
|---|---|---|---|---|
| | Maternal | Fetal | Maternal | Fetal |
| 1 | 90 | 200 | 130 | 1,290 |
| 2 | 43 | 480 | 180 | None |
| 3 | None | 167 | 120 | 755 |
| 4 | None | 110 | 180 | 840 |
| 5 | 85 | 320 | 205 | 1,080 |
| 6 | 92 | 455 | 240 | 645 |
| 7 | None | 133 | 165 | 690 |
| 8 | 77 | None | 148 | 1,120 |
| 9 | None | None | 180 | 1,200 |
| 10 | 82 | None | 128 | 1,100 |
| 11 | 150 | 320 | 260 | 1,430 |
| 12 | None | 570 | 185 | 670 |
| 13 | 88 | 227 | 134 | None |
| Mean* | 88.4 | 308.2 | 170.4 | 892.7 |

*"None" omitted.

**Table III.** Response of the fetal and maternal heart rates to cigarette smoking

| Case | Seconds to response | | Fetal anticipation |
|---|---|---|---|
| | Maternal | Fetal | |
| 1 | 245 | 164 | + |
| 2 | 125 | 92 | 0 |
| 3 | 152 | 83 | + |
| 4 | 220 | 140 | + |
| 5 | 114 | 235 | 0 |
| 6 | 200 | None | 0 |
| 7 | 103 | None | 0 |
| 8 | 110 | 240 | 0 |
| 9 | 260 | 320 | + |
| 10 | 110 | 360 | + |
| Mean* | 163.9 | 208.3 | |

*"None" omitted.

kilogram per minute for 4 minutes. There was a rise in maternal blood pressure with the expected slowing of the maternal heart rate. Fetal rate is unaffected except in one instance shown in Fig. 9 where as a result of uterine contractions there was marked fetal bradycardia.

*Frightening experience.* One patient was subjected to a frightening experience as follows: While the fetal heart rate was being recorded the anesthesiologist wheeled in a gas machine and began to inflate the bag and make adjustments on the mask. The maternal and fetal responses are shown in Fig. 10. The resultant maternal tachycardia was almost instantaneous. The fetal tachycardia followed very quickly with about the same order of time delay as shown with cigarette smoking.

This experiment is of particular importance not only because it shows the impact of emotional responses on the fetal heart but also in consideration of the causes of responses of the rapid type. Assali[10] has shown that the catechol amines do not cross the placenta and our experiments with epinephrine and norepinephrine although few in number would seem to confirm his findings. This being the case, the immediate responses of cigarette smoking and fright would seem to be initiated not by transfer of some substance across the placenta but more probably by a change in uterine blood flow.

## Conclusions

1. A reliable instantaneous fetal heart rate meter utilizing a phonocardiograph has been developed.

2. All of the data for this paper were obtained by crude measurements of paper tapes or semiautomatically from magnetic tapes. A more sophisticated method of data reduction is being developed.

3. Normal mothers were submitted to various stimuli to test their effect on maternal and fetal heart rate.

4. The responses to the inhalation of 100 per cent and 12 per cent oxygen were inconstant.

5. There was no fetal response to the administration of epinephrine or of norepinephrine.

6. Atropine produced vagal blockade and tachycardia in both the mother and the fetus. There was a significant time delay between the maternal and the fetal response. This was of such duration as to allow time for the drug to cross the placenta and blockade the fetal vagus.

7. Cigarette smoking produces fetal and maternal tachycardia. This response has a

short time delay and is similar to that produced by fright. It may be caused by a reduction in uterine blood flow.

8. It may be that accurate measurements of the parameters of such responses in normal and abnormal gravidas can serve as a test for fetal well-being and the adequacy of placental transfer.

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## Discussion

Dr. Carl P. Huber, Indianapolis, Indiana. Like many others, we have been interested in developing methods for the recording of the fetal heart. We believe that we have a very satisfactory method of monitoring the fetal heart during the second stage of labor and during that part of the first stage where the presenting part is available. The information which has been obtained demonstrates surprising variations in the heart rate associated with uterine contractions and intrauterine manipulations but with very questionable significance for marked decrease or irregularities in the fetal heart.

The present investigation is an entirely different approach to the study of the fetal heart rate in that it attempts to determine the response to a variety of stimuli on the part of the normal fetus during the latter part of pregnancy. The changes that have been produced by the exhibition of a variety of medication is on the whole not surprising. It is essentially consistant with our ideas concerning the physiologic responses of the fetus and the maternal-fetal placental relationships. It is to be hoped that further studies will in fact elucidate the ability of the normal fetus which may be in jeopardy.

It would seem that the degree of oxygenation of the fetus must be the deciding factor in response, or at least that what we are trying to measure in this type of study is the efficiency of the placental circulation. It is possible that methods of monitoring oxygenation of the intervillous blood or of measuring blood volume flow through the placenta may give us information that is a step or two ahead of the measurement of the fetal heart rate or of the fetal ECG.

All available data would indicate that we are on the threshold of devising or applying methods which will as accurately measure the status of the fetus in inner space as we can now measure the status of man in outer space.

It would seem that both respond to conditioned reflexes as evidenced in the fetus by the changes associated with offering a cigarette to the mother. It would also seem that both are remarkably well protected. Our information concerning them seems to depend upon our ability to feed the right taped data into the properly functioning computer.

Dr. Hellman (Closing). Fortunately, I had an opportunity to talk to Dr. Hunter about his apparatus and some of the difficulties he had in obtaining the very fine records which Dr. Huber has just shown you. As far as fetal electrocardiography is concerned, in order to attain records of the quality just exhibited, it is necessary to have a fetal scalp lead. Inasmuch as we needed intact patients and did not wish to rupture membranes, we elected to employ the phonocardiograph. This does not mean that the phonocardiograph is without its own particular and difficult problems.

# The pharmacologic control of excessive uterine activity with isoxsuprine

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THE great majority of neonatal deaths in this country occur in babies born prematurely.[1] Among the causes of this large group of perinatal deaths is the factor of excessive uterine activity; in some such instances, a presumably normal product of conception may be destined to die neonatally simply because labor is initiated prematurely.

In this particular group of pregnancies, the degree to which the onset of premature labor may be safely delayed should have a direct bearing upon the over-all perinatal mortality.

The obstetrician remains handicapped in his search for a means of preventing premature labor by his continuing lack of knowledge of the exact mechanism by which labor is initiated; for, despite great advances in the knowledge of the control of myometrial activity both in pregnancy and during labor, the precise determinants of the onset of labor remain nearly as much an enigma as ever. The clinician is further confused by the apparently paradoxical nature of the drugs in his armamentarium. For example, while morphine has long been employed to depress uterine activity,[2] it also on occasion appears clinically to restore rather promptly the clinical progress in a flagging labor. The confusion is compounded by the findings of laboratory observers that the commonly employed dosage of morphine has very little, if any, predictable effect upon the contractility pattern of the intact human uterus in late pregnancy and labor.[3, 4]

It has long been known that transient relaxation of the hyperactive uterus can be achieved through the use of small injections of epinephrine.[5] The transient nature of the effects achieved, however, together with the rather marked cardiovascular side effects, has sharply limited the usefulness of the role which epinephrine may play in clinical control of uterine hyperactivity. To be useful in the management of threatened premature labor, the ideal drug should have some of the effectiveness of epinephrine but greater duration of action, plus minimal unpleasant and/or unsafe side reactions. The present report concerns a study of a drug, isoxsuprine, which has some of these desirable qualities.

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***On leave from Department of Obstetrics and Gynecology, University of Nijmegen, Holland.*

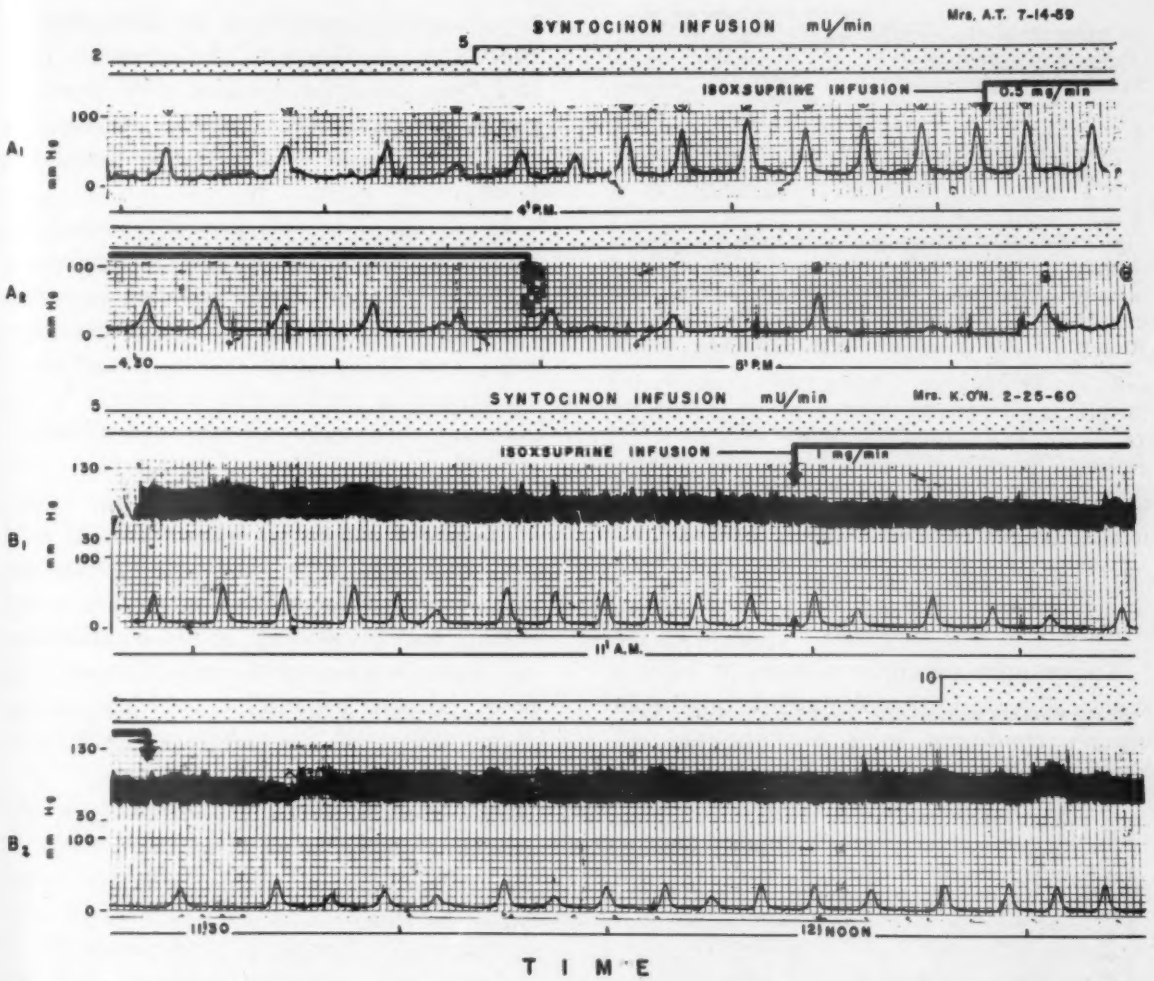


Fig. 1. *The effect of isoxsuprine on oxytocin-enhanced uterine activity.* $A_1$ and $A_2$, With the uterine activity being maintained by a constant infusion of 5 mU. per minute of synthetic oxytocin, an isoxsuprine infusion was begun and continued for 27 minutes at the rate of 0.5 mg. per minute. The sharp reduction of uterine activity was maintained after the isoxsuprine had been discontinued. $B_1$ and $B_2$, The good amount of oxytocin-sustained uterine activity was markedly reduced by an infusion of isoxsuprine at 1.0 mg. per minute. The isoxsuprine infusion brought about a mild but sustained reduction in the brachial arterial blood pressure. At the end of line $2_B$, it may be seen that when the oxytocin infusion rate was increased, the uterine activity increased promptly.

Isoxsuprine* is a drug of the β-phenylethylamine group of epinephrine-like compounds. It was synthesized by Moed and Van Dijk[6] after they had predicted on theoretical grounds prior to its synthesis that this compound ought to (1) be a vasodilator, (2) produce less tendency to tachycardia than does epinephrine, and (3) be effective when administered orally.

During a subsequent pharmacologic assessment of the potentialities of the drug, Lish, Dungan, and Peters[7] noted its uterine relaxant properties on animal uterine muscle strips and suggested that it should be further investigated for its possible uterine-relaxing properties in the intact human.

The mode of action of isoxsuprine is believed by Lish and his co-workers to be dominantly sympathomimetic, acting through beta adrenergic receptors. The additional suggestion is made, however, of an additional direct papaverine-like effect.[8]

Bishop and Woutersz[9] have just reported on a large clinical experience with isoxsuprine in cases of threatened premature labor. The present communication extends our

*2-(phenoxy-2-propylamino)-1-(*p*-hydroxyphenyl)-1 propanol hydrochloride (Vasodilan), Mead Johnson & Co.

Fig. 2. *Effect of isoxsuprine on spontaneous uterine activity.* Within 5 minutes after the start of the isoxsuprine infusion at 0.5 mg. per minute, the uterine activity dropped dramatically. A total of 10 mg. of the drug was given according to the schedule shown.

earlier report[10] on studies performed during the past 2 years, with greater emphasis on measurements of uterine and cardiovascular effects of the drug.

## Materials and methods

Thirty-eight pregnant women at various stages of gestation were studied under laboratory conditions in a constant-care observation unit situated on the labor floor of the University Hospitals of Cleveland. They had been admitted because they were already in active labor, or for induction of labor, or because of threatened premature labor.

In all cases, uterine pressures were continuously monitored through transabdominally placed intrauterine catheters by methods previously described.[11] Intrauterine pressures were taken from within the amniotic cavity and/or the intervillous space.[12] In the majority, there were also recorded the intra-arterial pressure (femoral or brachial) and the maternal heart rate. Maternal electrocardiograms were recorded in selected subjects. All data were recorded on Sanborn polyviso equipment.

Prior to the initiation of the transabdominal catheter placement, premedication was given, usually meperidine, 100 mg. intramuscularly, and meprobamate, 400 mg.,

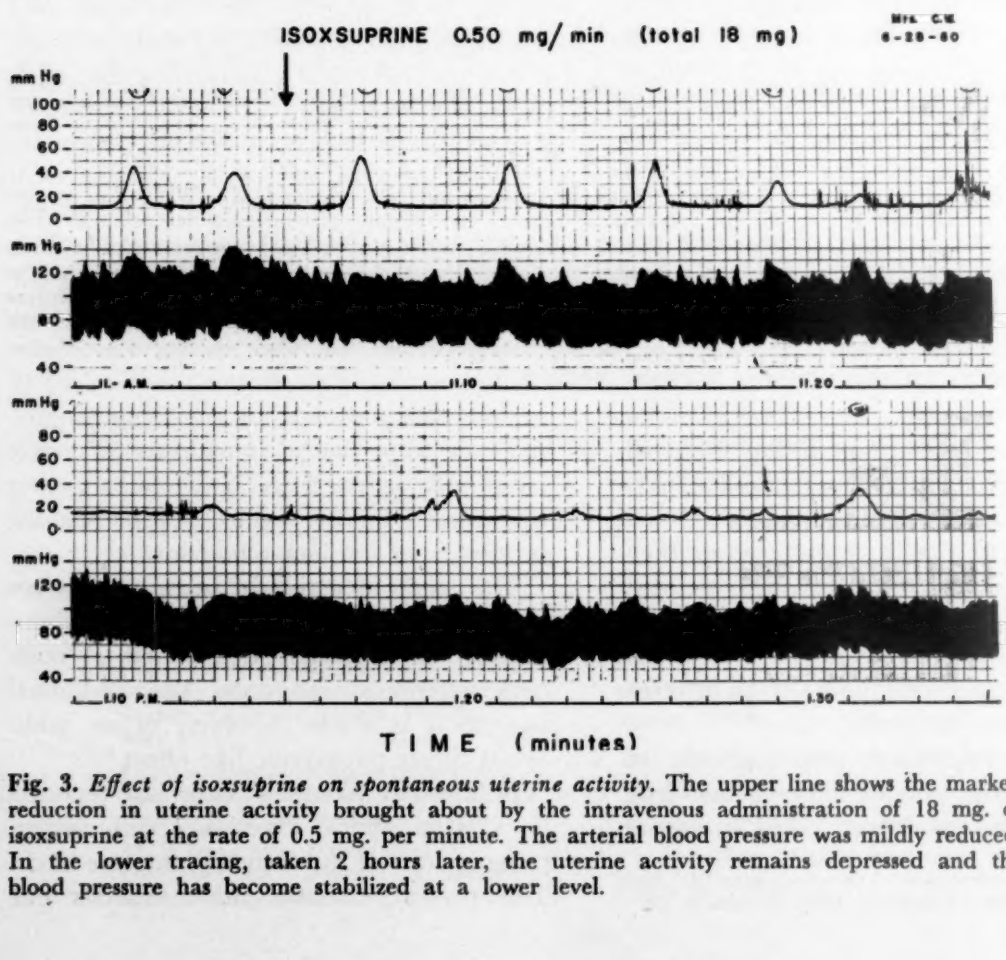


Fig. 3. *Effect of isoxsuprine on spontaneous uterine activity.* The upper line shows the marked reduction in uterine activity brought about by the intravenous administration of 18 mg. of isoxsuprine at the rate of 0.5 mg. per minute. The arterial blood pressure was mildly reduced. In the lower tracing, taken 2 hours later, the uterine activity remains depressed and the blood pressure has become stabilized at a lower level.

orally. Neither of these drugs has been demonstrated to have any depressant effect upon uterine activity in the dosage employed here. The remainder of the active study period, except for those cases in advanced active labor, was completed without any further sedation in almost every instance.

The effect of isoxsuprine was assessed both upon spontaneous uterine activity and upon activity which was being enhanced by constant pump infusions of synthetic oxytocin.*

## Results

The results of these studies will be reported under the following major headings: (A) General effect of isoxsuprine upon uterine contractility in late pregnancy, (B) Effect of the drug upon a small series of premature labors, and (C) Cardiovascular effects. Typical cases will be presented, following which a summarization will be made of the findings for the entire group.

**A. Effect upon uterine contractility in late pregnancy.** Twenty-five subjects (39 to 43 weeks' gestation) were included in this group. All had intact membranes. Seven were in spontaneous labor, while 18 were receiving infusions of synthetic oxytocin at the time isoxsuprine was first administered.

*Case 1.* This para viii was having labor induced 10 days after estimated date of confinement. The cervix was dilated 2 cm. Synthetic oxytocin was being administered intravenously by constant infusion pump at the rate of 2 mU. per minute at the beginning of the tracing shown in Fig. 1, *A*. At the point indicated on the chart, the oxytocin infusion rate was increased to 5 mU. per minute and the uterine activity became stabilized at a new, higher rate. The infusion of isoxsuprine at the rate of 0.5 mg. per minute brought about a clear reduction in both the intensity and frequency of the uterine contractions, the decrease starting to become evident within about 6 minutes after the infusion was begun. A total of 13.5 mg. of isoxsuprine was infused during 27 minutes. The decline in uterine activity continued throughout the infusion until the level of uterine activity,

*Syntocinon, Sandoz, Inc.

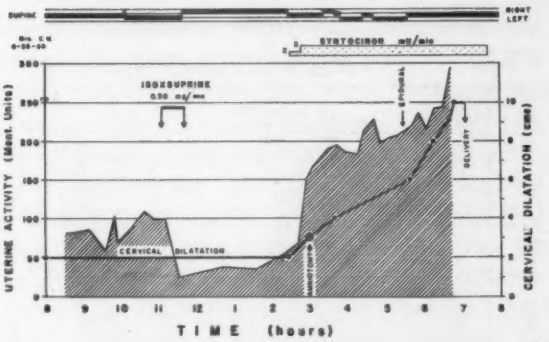


**Fig. 4.** *A 10 hour graphic summary of the clinical course of the patient whose tracings appear in Fig. 3.* The initial spontaneous uterine activity (striped area) was nearly 100 Montevideo units, compatible with that frequently present at the onset of spontaneous labor. The uterine activity was halved after the isoxsuprine infusion. Over 3 hours after the isoxsuprine administration was begun, small amounts of intravenously infused synthetic oxytocin plus amniotomy brought the subject into active labor.

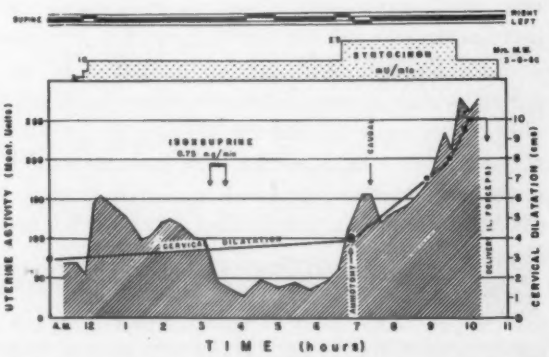


**Fig. 5.** *Effect of isoxsuprine infusion on uterine contractions in induced labor.* After a fairly stable level of uterine activity had been established by oxytocin infusion at over 100 Montevideo units, the isoxsuprine infusion sharply decreased the contractility for more than 3 hours, after which labor was again activated by amniotomy and increased dosage of oxytocin.

measured in Montevideo units,[11] had been reduced by nearly 75 per cent. Gradual recovery became evident one hour later, at which time the oxytocin infusion rate was raised to 10 mU. per minute, with a subsequent normal increase in uterine activity. The remainder of the induction was uneventful.

*Case 2.* This para vii, admitted at term for induction of labor, had a 2 cm. cervical dilatation and had been receiving synthetic oxytocin intravenously at 5 mU. per minute for over an hour before the initiation of isoxsuprine administration (Fig. 1, *B*). The uterine activity

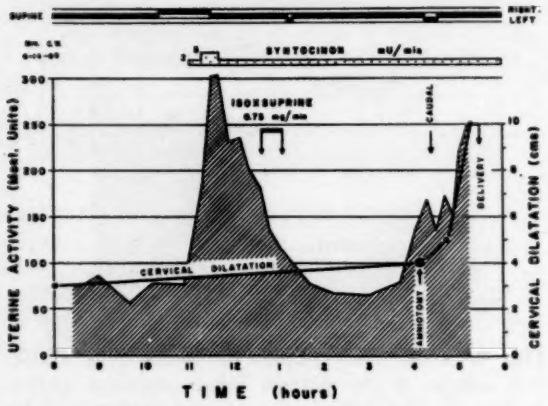


**Fig. 6.** *Clinical course of a woman in advanced prelabor who had developed an unusually great responsiveness to oxytocin.* Isoxsuprine infusion brought the uterine level back to the level existing before the oxytocin infusion had begun. After partial recovery of the uterine activity, amniotomy was performed and labor proceeded rapidly and normally.

was well stabilized. Twenty milligrams of isoxsuprine was infused at the rate of 1 mg. per minute. Reduction in uterine activity was observed within about 3 minutes after the beginning of the infusion, and the activity dropped by about 50 per cent, a level which was maintained fairly well for the next hour, at which time the oxytocin dosage was increased to 10 mU. per minute.

*Case 3.* This para iv was in advanced spontaneous prelabor at 39 weeks' gestation. The cervix was dilated 3 cm. There was no previous oxytocin. She received a total of 10 mg. of isoxsuprine intravenously over a 28 minute period (Fig. 2). The uterine activity showed significant reduction. Active labor was easily induced with synthetic oxytocin after a further 3 hour observation period.

*Case 4.* This para iv, with cervix dilated 2 cm. and in active prelabor at term, received a total of 18 mg. of isoxsuprine at the rate of 0.5 mg. per minute intravenously. As may be seen in Fig. 3, the uterine activity dropped dramatically. The clinical course is summarized in the chart of Fig. 4. The reduced uterine activity was maintained for over 3 hours, when labor was easily induced by amniotomy and the use of small amounts of intravenous synthetic oxytocin.

*Case 5.* This multipara was admitted to the hospital for induction of labor at term. The cervix was dilated 2 cm. As may be seen from Fig. 5, a fairly stable level of uterine activity was established through the infusion of 10 mU. per minute of synthetic oxytocin. When 20 mg. of isoxsuprine was infused at 0.75 mg. per minute, the uterine activity dropped to a level of less than 50 per cent of the previous values. Three hours later, active labor was easily induced by amniotomy and increasing the infusion rate of oxytocin. Labor and delivery were uneventful.

*Case 6.* This multipara was hospitalized at 41 weeks' gestation for elective induction of labor; the cervix was dilated 3 cm. She already was exhibiting a high degree of spontaneous uterine activity and was obviously in advanced prelabor. There was an unusually great sensitivity of the uterus to oxytocin, as demonstrated in Fig. 6 by the uterine responses to 2 and 5 mU. per minute of infused oxytocin. The oxytocin infusion rate was again dropped to 2 mU. per minute, and the uterine activity permitted to drop toward a lower level during the ensuing hour. At this point, isoxsuprine was infused at the rate of 0.75 mg. per minute for a total of 30 minutes. The uterine activity dropped more than 50 per cent. After 3 hours, amniotomy was performed, and delivery occurred a little over an hour later after an uneventful labor.

We believe that the cases just presented demonstrate beyond any reasonable doubt that isoxsuprine is capable of reducing the activity of the pregnant human uterus whether that activity is completely spontaneous or is being enhanced by intravenously administered synthetic oxytocin. In general, it may be said that the above cases illustrate the experience with this entire group of 25 subjects in late pregnancy with the exception of 2 factors. One of these factors is the degree to which active labor has already advanced by the time that the isoxsuprine administration is begun. In this series, for example, the 3 subjects who were in active labor and already dilated 5, 6, and 8 cm., respectively, before receiving isoxsuprine, exhibited only a fleeting uterine response to the drug in the doses given. The second limiting factor is that of dosage: the 3 subjects at term who received isoxsuprine at infusion rates of 0.25 mg. per minute had a minimal uterine response, especially inasmuch as the total dosages administered to

them were 7.5, 5.0, and 7.5 mg., respectively. In general, the larger the dosage, the more profound and prolonged the response of the uterus.

**B. Effect of isoxsuprine upon premature labor.** Thirteen cases of premature labor or threatened premature labor were studied. Of these, 4 cases had membranes ruptured prematurely and the remainder had intact membranes. In this group, all the uterine activity recorded was spontaneous (i.e., no oxytocin infusion studies were carried out). These patients ranged from 20 to 34 weeks' gestation.

*Case 7.* This subject had had 3 children born prematurely, of whom 2 were living. She was hospitalized in active labor at 29½ weeks' gestation. The cervix was dilated 4 cm., 50 per cent effaced, and the membranes remained intact. An isoxsuprine infusion of 30 mg. was given intravenously at the rate of 0.5 mg. per minute. The excessive uterine activity was promptly suppressed by the infusion (Fig. 7, *A*). One added 10 mg. dose of isoxsuprine was given intramuscularly during the night. The following day, the cervix was only 3 cm. dilated and the uterine activity remained under good control, as may be seen from the lower tracing of Fig. 7, *C*. The patient was discharged that day on oral isoxsuprine, 20 mg. four times daily. She was readmitted in labor 24 days following the first admittance; after a rapid labor, she was delivered of a healthy 1,956 gram female infant who has done well neonatally.

*Case 8.* This patient had had 3 previous premature infants. She was admitted at 29 weeks' gestation in active labor. The cervix was dilated 2 cm., 30 per cent effaced, and the

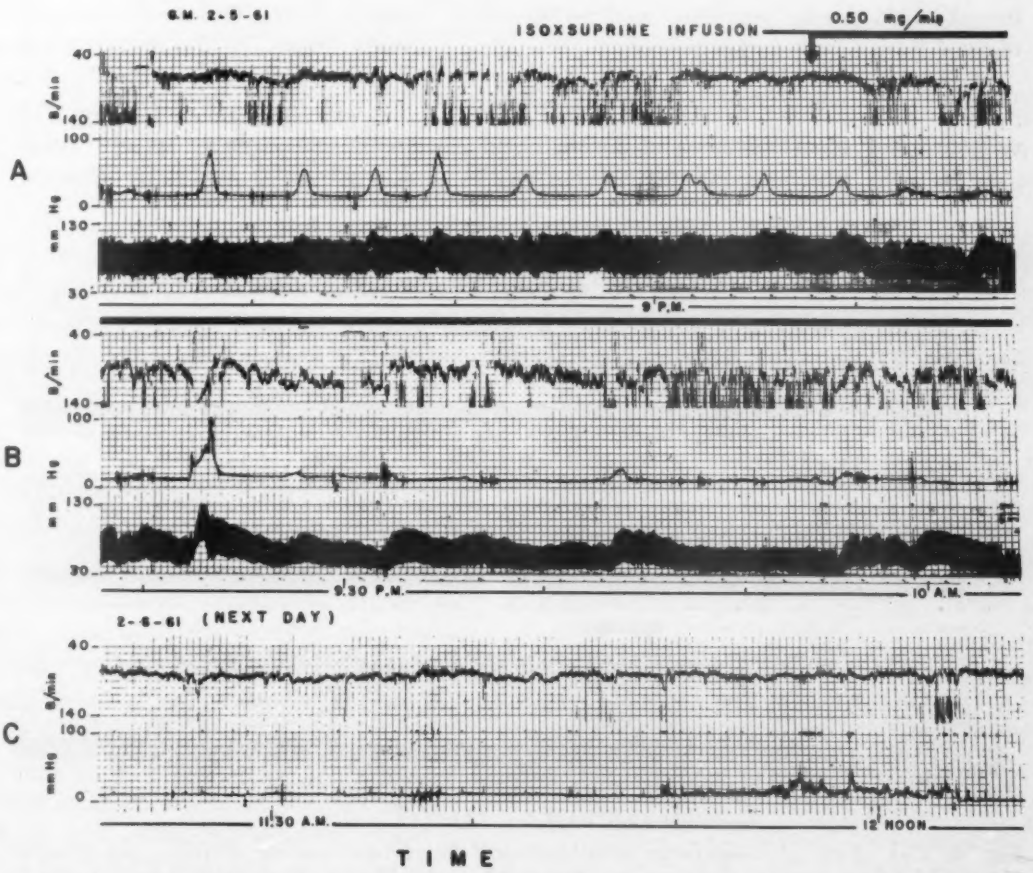


**Fig. 7.** *Effect of isoxsuprine in premature labor.* The top channel shows the maternal heart rate, the middle channel shows the uterine pressure record, and the bottom channel records the femoral arterial blood pressure. Lines *A* and *B*, After the infusion had begun, the uterine activity dropped promptly. The blood pressure drop and the tachycardia are well illustrated. Line *C*, The uterus remains quiescent. Delivery was delayed for 24 days.

membranes were intact. An unusually large amount of isoxsuprine was required to suppress the excessive uterine activity, a total of 57 mg. being infused during a period of 1½ hours (from about 6 P.M. to 7:30 P.M.) at rates varying from 0.5 mg. to 1.25 mg. per minute, according to the schedule detailed in Fig. 8. A further burst of uterine activity occurred at 9 P.M., and 10 mg. more of the drug was infused at 1.25 mg. per minute with good results. A further oral dose of 20 mg. was given later. A recording of the uterine activity on the following day (Fig. 9) shows how well the uterine activity was controlled. The patient was discharged on a schedule of 20 mg. of isoxsuprine four times daily. Eleven days later, the membranes ruptured spontaneously. The patient discontinued isoxsuprine therapy, and, after an uneventful labor, she was delivered of a 1,950 gram female infant. The infant was discharged from the "premature ward" of the pediatric service after 20 days, in apparent good health. The infant, delivered at 31 weeks' gestation, was the largest of the 4 infants born to this mother.

The remainder of the experience with premature labor is summarized in Table I. As may be seen, the attempts to prevent premature delivery after rupture of the membranes were notably unsuccessful. In all cases, some reduction in the spontaneous uterine activity was achieved. However, one 29 week fetus was delivered on the day of admittance, the infant succumbing to septicemia a few hours later. In the remaining 3 cases of this group, delivery was delayed by only 1, 2, and 5 days, respectively. In the 9 premature labors in which the membranes remained intact, the results were vastly more promising. Again in all cases, isoxsuprine brought about at least some evident reduction in uterine activity. In 3 of the 9 cases, however, delivery took place on the day isoxsuprine treatment was begun. In one of these, the 7 cm. dilatation of the cervix and the bulging membranes probably indicated that delivery was inevitable anyway. In 2 further cases, both studied at 3 cm. dilatation, delivery took place the same day as the study began. In the remaining 6 cases, labor was successfully arrested and delivery was delayed from 11 to 126 days. All these patients were treated by oral isoxsuprine, usually 40 to 80 mg. daily. Several were read-

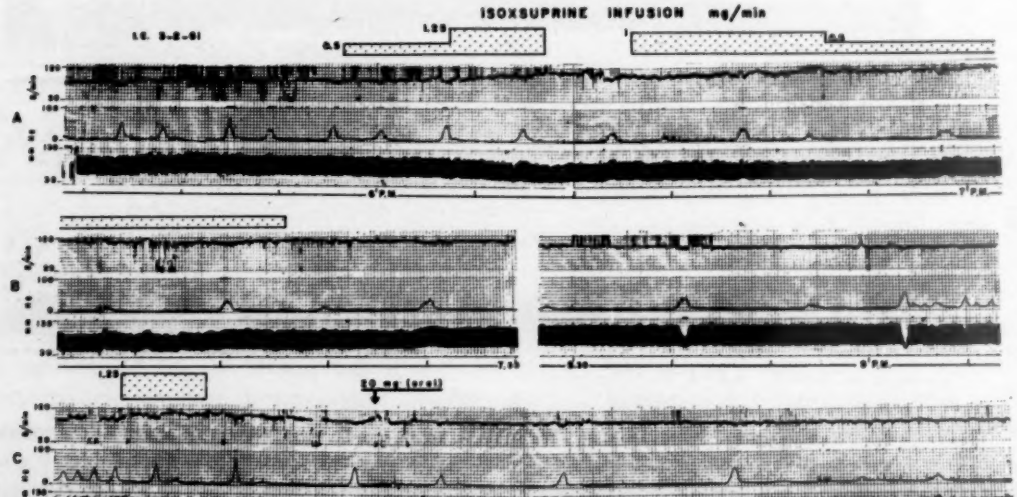


**Fig. 8.** ***The effect of isoxsuprine upon maternal heart rate, uterine activity, and femoral arterial blood pressure in a premature labor at 29 weeks.*** Line *A*, Isoxsuprine infusions significantly reduced the uterine activity and brought about some reduction in the femoral arterial blood pressure, as well as a mild tachycardia. When there was a burst of renewed uterine activity (end of line *B*) 10 mg. of isoxsuprine was given at an unusually high rate, 1.25 mg. per minute (line *C*). This dosage was supplemented by a further oral dose of 20 mg. and the uterine activity continued to subside.

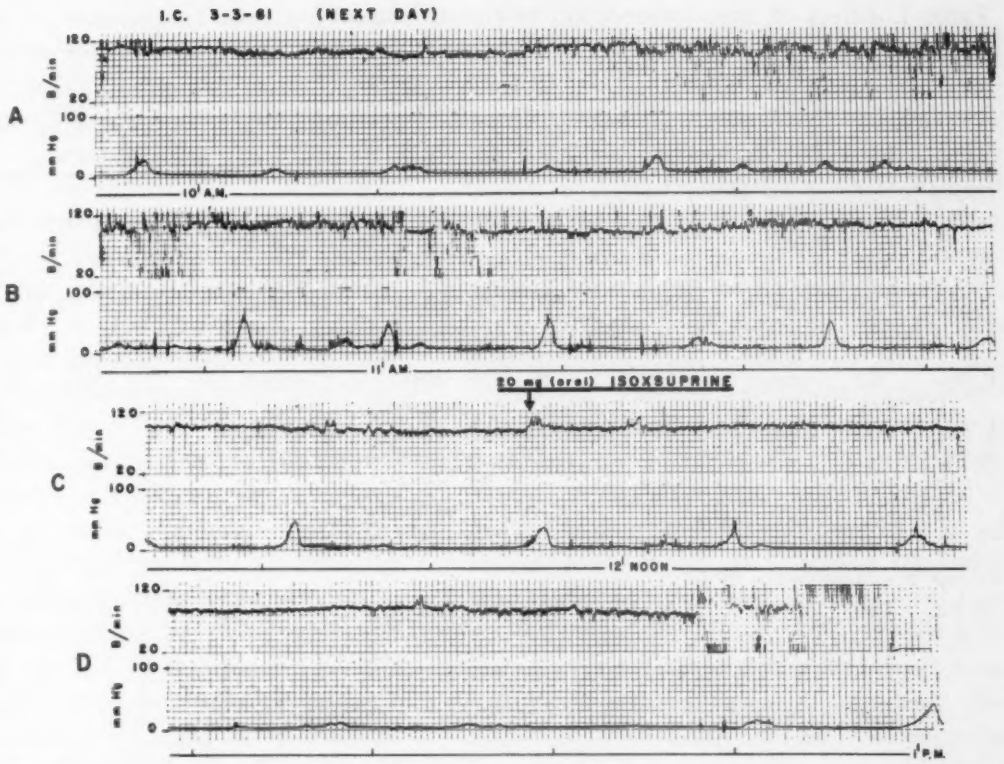


**Fig. 9.** *Observations on the same patient as in Fig. 8. Made on the following day.* The uterine activity remained markedly reduced, and the patient was receiving isoxsuprine orally. In line *D* is the response to 20 mg. isoxsuprine administered 30 minutes before. Labor was delayed for 11 days.

mitted for more intensive (intramuscular or intravenous) therapy.

**C. Cardiovascular and other side effects of isoxsuprine.** In view of its structural relationship to epinephrine, it should not be surprising that isoxsuprine should have some effects upon the cardiovascular system.

The most consistent side effects following the parenteral administration of isoxsuprine are in the cardiovascular area. The alterations in blood pressure and heart rate appear to vary somewhat with the route of administration and the rate at which the drug is being administered. As may be seen in Table II, in subjects receiving isoxsuprine intravenously within the dose range of 0.25 to 0.50 mg. per minute, 22 out of 23 exhibited a mean drop in systolic and diastolic blood pressures of 20 and 12 mm. Hg, respectively. In this group, the mean heart rate increased by 20 beats per minute. These changes were effected within about 6 minutes, and in all but one case the blood pressure had returned to its approximate original values within 1½ hours after the infusion was discontinued. In a single one of these 22 cases, the blood pressure drop was more severe, failing to re-

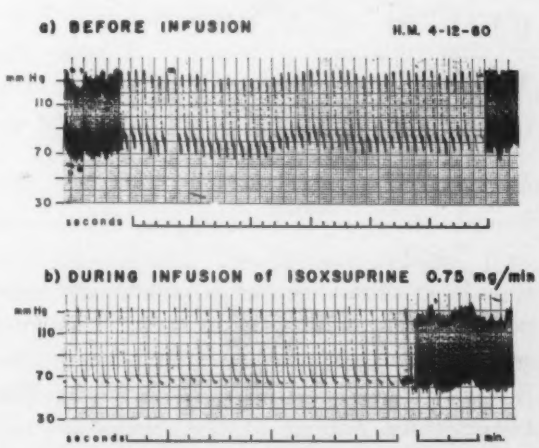


**Fig. 10.** *Effect of isoxsuprine on the arterial pressure wave. a,* Before the infusion; the reflected pressure waves are quite distinct. *B,* During the infusion of oxytocin; the reflected pressure waves cannot be seen, even when the tracing is being made at a higher speed.

**Table I.** Effect of isoxsuprine upon premature labor

| Subject | Weeks | Labor | Cervix: Dilatation (cm.) | Cervix: Effacement (%) | Isoxsuprine given first 12 hours (mg.) | Decrease in uterine activity | Days gained | Weight of baby (grams) | Outcome for baby |
|---|---|---|---|---|---|---|---|---|---|
| *Group A—Membranes already ruptured* | | | | | | | | | |
| C. Gl. | 28 | Active | 3 | 90 | 60, intravenously | Moderately large | 2 | 1,680 | Home, well at 31 days after repairing hernia |
| T. Gr. | 29 | Moderately active | 2 | 50 | 30, intramuscularly | Transient | 0 | 1,790 | Died first day, septicemia |
| E. Be. | 33 | Active | 4 | 80 | 20, intravascularly | Transient | 1 | 1,640 | Home, well at 28 days |
| J. Pa. | 33 | Early | 1 | Uneffaced | 32, intravenously<br>10, intramuscularly<br>40, orally | Moderate | 5 | 1,650 | Home, well at 25 days |
| *Group B—Membranes intact* | | | | | | | | | |
| M. Sw. | 20 | Active | 1 | 50 | 58, intravenously | Marked | 126 | 3,060 | Delivered by cesarean section at 38 weeks for cord prolapse. Home, well at 9 days |
| L. Wa. | 28 | Active | 3 | 70 | 30, intravenously | Moderate, transient | 0 | 1,170 | Home, well at 77 days |
| G. Mi. | 29½ | Active | 4 | 50 | 30, intravenously<br>10, intramuscularly | Moderately large | 24 | 1,956 | Home, well at 20 days |
| I. Cl. | 29 | Active | 2 | 30 | 67, intravenously<br>20, orally | Large | 11 | 1,950 | Home, well at 20 days |
| O. Na. | 31 | Hard, membranes bulging | 7 | 80 | 20, intravenously | Transient, mild | 0 | 1,430 | Died at age 3 days. Autopsy showed no abnormality |
| E. Jo. | 32 | Active | 2 | | 32, intravenously | Marked | 23 | 2,610 | Home, well at 3 days |
| M. Ha. | 32 | Active | 3 | | 31, intravenously | Transient | 0 | 2,105 | Home, well at 14 days |
| C. Wi. | 33 | Early | 1 | 10 | 30, intravenously | Moderate | 19 | 2,600 | Home, well at 3 days |
| E. Pi. | 34 | Very active | 4 | 50 | 29, intravenously | Moderate | 20 | 2,350 | Home, well at 3 days |

spond to Trendelenburg positioning and pressor agents. In this instance, the blood pressure was effectively restored by the infusion of 5 mU. per minute of oxytocin. The twenty-third patient of this group did not exhibit any observable changes in blood pressure or heart rate.

Among 17 observations with infusions of isoxsuprine within the range of 0.75 to over 1 mg. per minute, 16 showed the same type of cardiovascular changes, while the other one showed no blood pressure drop. In the 16 who showed some blood pressure reaction, the mean systolic and diastolic drops were each 15 mm. Hg, while the mean increase in heart rate was 28 beats per minute. These changes had reached their maximum levels within 8 minutes; again the blood pressure had fully recovered within 1½ hours after the drug infusion was discontinued.

Comparable responses were seen in a small series treated by intramuscular injections of isoxsuprine, the principal difference being that the maximum effects were not evident until about 15 minutes after intramuscular administration, and the recovery of the blood pressure level was somewhat delayed. In all groups listed in Table II, the relative tachycardia tended to persist for some time after the recovery of the blood pressure.

Typical blood pressure and/or heart rate responses to isoxsuprine may be seen in Figs. 1, *B*, 3, 7, 8, and 9.

The significant cardiovascular effects appear not only to be associated with progressive uterine relaxation but also to be compatible with the findings to be expected in generalized vasodilatation. An interesting piece of evidence along this line appears in Fig. 10. The top half of this figure (line *a*) is a tracing of the femoral arterial pressure made prior to the beginning of an infusion of isoxsuprine. A well-defined reflected pressure wave is evident in the diastolic phase of each contraction. In line *b*, however, when the tracing was taken during an infusion of isoxsuprine, the reflected pressure wave has almost entirely disappeared. The suppression of the reflected pressure waves persists until both the blood pressure and the heart rate have returned to normal levels. The absence of such reflected waves may be due to a large degree of dilatation of the peripheral arterioles, a situation which can be induced by vasodilator drugs.[13]

Electrocardiogram tracings showed no significant changes in response to isoxsuprine.

Side effects other than those discussed above have been noted in some instances. Perhaps the most common symptom is nausea, but this is usually of transient duration. Occasional sweating is seen. A number of the subjects complained of drowsiness (unassociated with any severe hypotension), and several have gone soundly to sleep during the infusions. Two subjects complained briefly of frontal headaches.

No untoward effect on the fetal heart rate was observed in any subject that was studied.

## Comment

In the control of excessive amounts of uterine activity in human pregnancy, isoxsuprine is not the "ultimate drug," but only the latest and, to date, probably the most effective in the management of threatened premature labor.

The "ideal drug" should have no side reactions. Isoxsuprine *does* have side reactions. Most of these side reactions are of little moment: the mild nausea, sweating, and occasional headache represent little more than minor discomforts to a patient who is most anxious to continue to carry her pregnancy successfully.

The most significant side reaction is, of course, hypotension. It is almost inevitable that successful treatment of threatened premature labor with isoxsuprine will be associated with mild lowering of the blood pressure because of the nature of the drug. The degree of hypotension induced by isoxsuprine is not wholly dependent upon the total amount and the rate at which the drug is given. It also depends upon the state of the patient at the time it is administered. We have found that we can minimize the hypotensive response by having the patient turned to the lateral rather than the supine position during the infusion. Again, as might have been anticipated, the well-hydrated subject appears to tolerate the drug better than the partially dehydrated one. Finally, the potential cumulative circulatory effect of other drugs given to the same patient must be borne in mind.

The first few minutes after the initiation of parenteral isoxsuprine therapy is the time when the patient's cardiovascular response can be assessed. The maximum blood pressure drops usually appear within 6 minutes when the drug is being given intravenously and within 15 minutes when it is administered by the intramuscular route. Particularly vigilant observation of the blood pressure and heart rate for a short time after parenteral therapy begins should help to insure against excessive dose rates. We believe that, while parenteral isoxsuprine therapy may often be indicated in the early man-

**Table II.** Effect of parenterally administered isoxsuprine on blood pressure and heart rate

| | *Total observations* | *No. showing blood pressure drop* | *Mean blood pressure drop (systolic/diastolic)* | *No. showing tachycardia* | *Mean increasing heart rate (beats per minute)* | *Time for maximum effect* |
|---|---|---|---|---|---|---|
| Intravenous, 0.25 to 0.50 mg. per millimeter | 23 | 22 | 20/12 | 22 | +20 | 6 minutes |
| | (Blood pressure recovery in 22 cases within 1½ hours, tachycardia still present.) | | | | | |
| Intravenous, 0.75 to 1+ mg. per millimeter | 17 | 16 | 15/15 | 17 | +28 | 8 minutes |
| | (Blood pressure in all recovered during 1½ hours, tachycardia still + 20.) | | | | | |
| Intramuscular, 10 mg. | 5 | 4 | 20/15 | 2 | -- | 15 minutes |
| | (Recovery within 2 hours, tachycardia still present.) | | | | | |

agement of premature labor, the parenteral administration of the drug should in most cases be reserved for the hospitalized patient. The oral form of the drug (at least to 80 mg. per day in divided doses) appears safe for use in the ambulatory patient, since it was used for months in some women without untoward effects. When the drug is given by any route, the patient should be kept under careful observation until it is clearly evident that she will tolerate the dosage schedule.

The total cardiovascular response to intravenous infusions of isoxsuprine is amazingly similar to that seen in response to infusions of epinephrine (when epinephrine is used in physiologic dosage, 2 to 10 μg per minute) in the time of induction of the maximal changes in blood pressure and heart rate, the pattern of change, and the pattern of recovery.[14] The effect on uterine activity, while initially similar to the effect of epinephrine, is far more prolonged.

There are 3 principal types of situations in which isoxsuprine has been observed to be ineffective:

1. When the drug is used in too small dosage to be effective. This point is too obvious to need further comment. It may be added, however, that the duration of the infusion also appears to be a significant factor. When isoxsuprine is being given by the intravenous route, we have found that the infusion should be maintained if possible for at least 1½ hours in order to bring the uterine activity down to a more satisfactory level and to maintain it there long enough for it to be effective in suppressing premature labor.

2. When the membranes are already ruptured. Initial dosage with isoxsuprine in such instances will produce a lowering of the uterine activity. But rupture of the membranes is the most effective single device for the induction of labor. Thus, even though uterine activity can be depressed with initial dosages of isoxsuprine, the same factor which enhances uterine contractility apparently continues to operate, and thus uterine activity increases again unless isoxsuprine therapy is pursued continuously and vigorously. We suspect that such vigorous therapy could help to carry some pregnancies for prolonged periods of time after rupture of the membranes. However, this is not usually a very practical procedure, because of the dosage schedule involved, the nursing care required, and, probably most important, the danger of intrauterine infection with a prolonged period after rupture of the membranes.

As a final point, it may be pointed out

that intrauterine infection, like active infection of the renal excretory tract, tends to increase uterine activity and thus the tendency toward premature delivery. We have observed one case of uterine hyperactivity associated with acute pyelonephritis. In this instance, an unusually large infusion rate of isoxsuprine was needed to reduce the uterine activity significantly and the recovery of uterine activity after the drug was discontinued was unusually rapid.

3. When given late in labor, the drug has no more than a transient effect. This is not to be wondered at, inasmuch as, once normal labor is set in motion, the normal course of events is that of constant increase in the effectiveness of labor and of cervical dilatation. There is a "point of no return" beyond which any given drug will not be effective within a given dosage range. With isoxsuprine, it appears that when labor has progressed until the cervix is dilated 5 cm. or more the likelihood of arresting labor with the usual dosage schedule of isoxsuprine is small.

The biggest question of all is this: Granted that a significant proportion of premature births may become preventable or delayed by the use of drugs such as isoxsuprine, how great is the potential gain in useful human lives which may be brought about by the prevention of premature birth alone? The implications of this question go far beyond the scope of the present paper.

**Summary**

1. Isoxsuprine, an epinephrine-related drug, was studied in 38 pregnant human subjects.

2. In 25 subjects in late pregnancy and/or labor, isoxsuprine was shown to reduce uterine activity in varying degrees.

3. In 13 individuals in premature labor, uterine activity also was reduced by isoxsuprine. Of the 4 subjects with ruptured membranes at the time the study began, labor was not delayed significantly, the longest "time gain" being 5 days. In the 9 subjects in premature labor with intact membranes, 6 pregnancies were prolonged significantly, the range being from 11 to 126 days.

4. Modest reductions in blood pressure and rises in heart rate accompany the administration of isoxsuprine by the parenteral route. The cardiovascular changes are compatible with those found in a generalized vasodilatation. No effect of isoxsuprine in the electrocardiogram was observed.

5. Prolonged depression of uterine activity could not be demonstrated in individuals already in advanced labor or where the membranes had been ruptured.

6. No alterations in the fetal heart rate were observed during isoxsuprine therapy.

7. The drug shows promise of genuine clinical usefulness for the suppression of threatened premature labor where the membranes remain intact.

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## Discussion

Dr. S. Leon Israel, Philadelphia, Pennsylvania. It is not difficult to open the discussion on such a paper, particularly since Dr. Hendricks sent me a copy well in advance of this meeting. It is, however, not easy to find something to discuss critically when the work reported has been painstakingly performed and cautiously interpreted. The points to be raised in my remarks are based not only upon the observations of Dr. Hendricks and his collaborators but also on the clinical investigations of 2 of my colleagues at the Pennsylvania Hospital, Dr. Edward H. Bishop, to whose preliminary report with Woutersz on isoxsuprine the essayist alluded,[1, 2] and Dr. Paul E. Stroup,[3] who has recently further refined our knowledge concerning the myometrial-inhibiting effects of epinephrine. This discussion will be limited to three items: a brief appreciation of the casualness with which we have come to accept such difficult medical research, some comments upon the contractility-arresting property of isoxsuprine, and a few remarks concerning the scope of measures to control unwanted uterine activity in the projected conquest of prematurity.

It is fruitful to reflect upon the seemingly effortless way in which isoxsuprine came to be synthesized. It was, as Dr. Hendricks related, "predicted on theoretical grounds" that such a contrived compound ought to be a good orally administered vasodilator free from cardiac effects. Similarly, it is of interest to meditate upon the aggregation of physiologic data that we have come to regard as commonplace in clinical research. We bring to our listening,. or our reading, such automatic anticipation that the "polyviso recordings," "multiple parameters," and "a battery of observations" are in danger of becoming hackneyed.

The mechanism by which isoxsuprine suspends uterine contractility must be by direct action on the myometrium at molecular level. Isoxsuprine does not work by suppressing oxytocin production for, as Dr. Hendricks has just shown us, the inhibition overrides simultaneously administered oxytocin. Its muscle-quieting action cannot be attributed either to its vasodilating property or to some effect upon the placenta's "progesterone block" inasmuch as Lish, Hillyard, and Dungan[4] have shown isoxsuprine to be effective in vitro on strips of human myometrium. Whatever the mechanism, the power of isoxsuprine to arrest spontaneous, as well as induced, contractions of the term uterus has been well illustrated by Dr. Hendricks and his co-workers. Moreover, there seems little doubt, from the apparently matched results of the research reported today and that of Bishop that isoxsuprine offers a 2 to 1 chance to arrest premature uterine contractions if the cervix is not widely dilated and if the membranes are intact. The duration of the inhibition, much longer than that achieved by epinephrine, and its relative freedom from associated side effects indicate an obvious biologic utility and heighten its practical use. It should, however, be reaffirmed—especially for those eager to apply it to their prematurely parturient patients—that Dr. Hendricks, as well as Bishop, emphasized that isoxsuprine is likely to be clinically ineffective when given in insufficient quantity if the lowered uterine activity is not maintained for at least 90 minutes, in the presence of ruptured membranes, and after cervical dilatation has reached 4 or 5 cm.

The last facet of this discussion goes beyond the aim of today's report on the efficacy of isoxsuprine to arrest uterine contractions to the point of achieving viable fetal maturity. There is universal agreement that such pharmacologic, stopgap measures are invaluable in their fashion. Fetal survival *is* linked directly to birth weight, neonatal mortality dropping sharply as 1,500 grams or more is attained. It is as ancient as folk tales to know that prematurity is deleterious to childhood, cheating the world of healthy adults. Noting this, Shakespeare's crippled Richard III complained of himself, "I that am rudely stamped, . . . deformed, unfinished, sent before my time into this breathing world, scarce half made up. . . ." Knobloch and Pasamanick[5] put it more academically, though perhaps less poetically: "At a comparable age premature infants, on the whole, are ½ to 1 inch shorter and 500 to 1,000 grams lighter, have 2 to 3 times as many physical defects, and have 50 per cent more illnesses than full-term control infants." It is obvious that our major effort must be to prevent the *onset* of premature uterine contractility. The unknown causes of prematurity must be attacked vigorously by a world-wide program designed to correct all errors of maternal nutrition, to identify deficiencies of uterine circulation during pregnancy, and to interpret the etiological meaning of such maternally tolerated poisons as nicotine and bacterial toxins.

Finally, may I conclude by offering Dr. Hendricks and his team congratulations upon the execution of a carefully planned clinical experiment and by expressing admiration for their restraint. They have resisted the temptation described by Samuel Butler, "To draw sufficient conclusions from insufficient premises."

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Dr. David N. Danforth, Evanston, Illinois. There is no question that the major problem in obstetrics is the control of uterine motility. Dr. Hendricks' studies in recent years have done much to clarify this problem, and it seems clear that the ultimate solution will evolve from the further pursuit of the particular studies upon which he is now engaged. The present study is an especially exciting one since in addition to its clinical value we now have a new drug which has predictable and reproducible effects upon the uterus.

The failure of isoxsuprine to act after labor is established emphasizes how very complex the control of uterine action is. Twenty-five years ago I became intrigued with Blair Bell's work upon the relation of calcium ions to uterine contractions, and in 1949 I wrote what then seemed to me a tour de force on the subject of the cause of the onset of labor. Although the conclusions were somewhat premature, it did seem to me inescapable that the ultimate control of uterine motility was based upon changes in electrolyte environment. Curiously, modern work seems to support this conclusion. There is good evidence that the ovarian hormones profoundly affect mineral metabolism and, more recently, they are shown to modify the electrolyte patterns of uterine muscle. It is clear that the actomyosin-adenosine triphosphate complex requires a specific and optimal electrolyte environment for its proper action. And it is probable that the incoordinate uterine action of experimental diabetes insipidus is basically an electrolyte defect. These, and many other observations, strengthen the likelihood that, when the final word is written, the electrolyte environment of the uterus will be found to be the basic factor in the control of uterine action. Certainly, each facet of Dr. Hendricks' work brings us one step closer to this utopian circumstance when it will be possible to control the uterus at will. I should like to ask Dr. Hendricks if at this stage of his work he can suggest the means by which isoxsuprine exerts its uterine effects. Is it possible that this is at the myometrial cell level? Also, is this uterine effect similar to that of magnesium? Finally, he has shown dramatic effects of oxytocin in relieving the vascular collapse which followed isoxsuprine in one case. Might oxytocin be useful in other cases of obstetric shock?

In closing, I should like to congratulate Dr. Hendricks most warmly upon this exciting and beautifully executed study.

Dr. Hendricks (Closing). No one has yet seriously suggested that isoxsuprine acts centrally. The primary action site is considered to be the so-called "beta adrenotropic receptor," located, according to Ahlquist's original report, "in, on, or near the muscle or gland cells affected by epinephrine." Most of the pharmacologic characteristics of the drug appear to be compatible with an action produced by stimulation of the beta receptor of the smooth muscle cell. However, Lish continues to feel that an additional direct papaverine-like effect is suggested by the results of animal experimental work, but the importance of this action on the human myometrium has not yet been established.

Dr. Danforth has asked why one patient's severe hypotension was effectively counteracted by the infusion of a small amount of oxytocin. This was in a multiparous individual, in early spontaneous labor, who was probably somewhat dehydrated, and whose systolic blood pressure exceeded 100 mm. Hg only with uterine contractions during the initial observation period before any infusion was started. When isoxsuprine infusion was begun at 0.5 mg. per minute both the uterine activity and the blood pressure dropped, but the blood pressure was seen to be always restored again almost instantaneously to normal levels by even modest uterine contractions. Sustained recovery of the blood pressure was brought about after the uterine activity was raised (by the administration of 5 mU. per

minute of oxytocin by pump infusion) to levels like those seen in normal active labor (about 200 Montevideo units). Our tentative explanation of this phenomenon is this: When normally frequent uterine contraction cycles were restored, the extrusion of 200 or 300 c.c. of blood from the uterus at the beginning of the contraction augmented the effective circulatory volume sufficiently to restore a more adequate circulation. The patient experienced no further difficulty and delivery of a normal infant took place about 3 hours later.

# Maternal-fetal oxygen and acid-base studies and their relationships to hyaline membrane disease in the newborn infant

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THIS report is concerned with the results of simultaneous measurements of oxygen tension, acid-base balance, and hydrogen ion concentrations in the pregnant mother and the fetus at the moment of birth. These biochemical measurements, obtained in patients with uncomplicated gestation, are compared to similar ones found in women whose pregnancies were disturbed by disease. The latter complications in this study include pre-eclampsia, diabetes, and multiple gestation. The purpose of this investigation is to demonstrate a possible relationship between the alterations in oxygen, carbon dioxide, bicarbonate, and pH equilibria found in the latter group and the occurrence of hyaline membrane disease in the newborn child.

## Material and methods

All observations were made on patients at the Colorado General Hospital. The clinical data on 4 patients without complications and 6 gravidas with complications of pregnancy are outlined in Tables I and II. The techniques employed for obtaining blood from the intervillous space and umbilical vessels were the same as those described by Prystowsky.[1] Radial artery blood was collected by direct arterial puncture. Plasma pH was measured on a Beckman pH meter which reads to within ±0.02 pH units and was standardized with a phosphate buffer of known hydrogen ion concentration. The pH determinations were made at 37° C. in a constant temperature room. The oxygen and carbon dioxide contents of whole blood and plasma were determined by the method of Van Slyke and Neill.[2] Tonometer gases were measured by the method of Scholander.[3] Calculations for the blood gases were the same as those previously reported.[4] The partial pressure of oxygen was obtained by referring the per cent of oxygen saturation (oxygen content divided by oxygen capacity times 100 equals per cent saturation) to an oxygen dissociation curve, constructed at the actual pH of the mother and fetus and measured at this altitude of 5,280 feet.[5] The oxygen and carbon dioxide gradients were determined by subtracting the umbilical vein and artery pressure from the intervillous space pressure ([IVS-UV] + [IVS-UA] divided by 2 = gradient). Blood samples for determination of maternal and fetal standard bicarbonate were drawn simultaneously with the other maternal and fetal samples. Standard bicarbonate was determined at a $CO_2$ pressure of 40 mm. Hg, temperature of 37°

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C., and whole blood fully oxygenated; the technique of obtaining this value was reported.[6]

## Results

**Oxygen data, uncomplicated pregnancy.** All oxygen measurements from patients with uncomplicated gestation are presented in Table III. The mean oxygen content of the radial artery is 16.1 volumes per cent. At the intervillous space, the mean oxygen content was 12.6 volumes per cent. The difference between the radial artery and intervillous space oxygen content was 3.5 volumes per cent. The umbilical vein contains 11.4 volumes per cent while the blood returning from the fetus to the placenta via the umbilical artery contained 5.6 volumes per cent. The arteriovenous oxygen difference across the fetal circulation was 5.8 volumes per cent. The oxygen saturation in the maternal artery averaged 95.2 per cent. This figure is within the reported range for normal pregnant and nonpregnant subjects. The intervillous space oxygen saturation averaged 75 per cent ($O_2$ pressure, 42.9 mm. Hg), the umbilical vein, 54 per cent ($O_2$ pressure, 22.5 mm. Hg) and the umbilical artery, 26.6 per cent ($O_2$ pressure, 13.9 mm. Hg). The oxygen pressure difference between mother and fetus, or the oxygen gradient, in uncomplicated pregnancy averaged 24.7 mm. Hg. The range of variation for these values is wide and agrees with previous data.[7]

**Oxygen data, complicated pregnancy.** Oxygen data in abnormal pregnancy are shown in Table IV. The radial artery oxygen content in the abnormal group averaged 16.1 volumes per cent, the same as in the controls. The simultaneous intervillous

**Table I.** Clinical observations on 4 mothers whose pregnancies, labors, and deliveries were uncomplicated

| *Case No.* | *Age (years)* | *Parity* | *Duration of pregnancy (weeks)* | *Condition* | *Anesthesia** | *Method of delivery* | *Infant weight in grams and condition* |
|---|---|---|---|---|---|---|---|
| 1 | 18 | 1 | 40 | Previous section | Spinal | Abdominal | 2,450, normal |
| 2 | 17 | 2 | 39 | Previous section | Spinal | Abdominal | 2,920, normal |
| 3 | 18 | 1 | 39 | Normal | Local | Vaginal | 2,970, normal |
| 4 | 20 | 1 | 39 | Previous section | Epidural | Abdominal | 2,670, normal |

*None of the patients in this group received oxygen.

**Table II.** Summary of clinical findings on 6 patients with complications of pregnancy

| *Case No.* | *Age (years)* | *Parity* | *Duration of pregnancy (weeks)* | *Complication* | *Anesthesia and method of delivery* | *Apgar score** | *Infant weight in grams and condition* |
|---|---|---|---|---|---|---|---|
| 5 | 27 | 4 | 38 | Unsuccessful induction, diabetes | Spinal, abdominal | 7 | 3,510, hyaline, x-ray, lived |
| 6 | 20 | 0 | 36 | Previous section, diabetes | Spinal, abdominal | 9 | 3,270, normal, lived |
| 7 | 20 | 0 | 35 | Toxemia, twins (fraternal) | Caudal, vaginal | 4<br>4 | A-2,080, hyaline, lived<br>B-2,380, hyaline, lived (imperforate anus) |
| 8 | 33 | 5 | 33 | Previous section, twins, labor (identical) | Spinal, abdominal | 9<br>4 | A-1,800, hyaline, lived, nasal pneumococci<br>B-2,200, hyaline, x-ray, died, autopsy |
| 9† | 28 | 1 | 37 | Previous section, toxemia | Spinal, abdominal | 9 | 3,190, normal, lived |
| 10 | 20 | 0 | 35 | Toxemia, twins (identical) | Local, vaginal | 4<br>4 | A-1,960, hyaline, lived<br>B-2,100, hyaline, lived |

*Apgar score at the moment of birth based on a score of 0-2 for each of the following: (a) heart rate, (b) respiratory effort, (c) muscle tone, (d) reflex irritability, and (e) color.

†In Case 9, the patient received 100 per cent oxygen before delivery.

**Table III.** Individual and average values for maternal and fetal oxygen in uncomplicated pregnancies

| *Case No.* | *Vessel** | *pH at 37° C.* | *$O_2$ content (vol. %)* | *$O_2$ capacity (vol. %)* | *$O_2$ saturation (%)* | *Estimated $O_2$ partial pressure (mm. Hg)* | *Estimated $O_2$ pressure difference between mother and fetus (gradient) (mm. Hg)* |
|---|---|---|---|---|---|---|---|
| 1 | RA | 7.44 | 14.3 | 15.2 | 94.1 | ---- | |
| | IVS | 7.43 | 13.6 | 15.2 | 89.9 | 60.0 | |
| | UV | 7.42 | 12.3 | 19.1 | 64.5 | 24.0 | 41.0 |
| | UA | 7.41 | 5.1 | 19.1 | 26.8 | 14.0 | |
| 2 | RA | 7.43 | 15.5 | 16.3 | 94.6 | ---- | |
| | IVS | 7.44 | 10.0 | 16.3 | 61.5 | 31.5 | |
| | UV | 7.44 | 5.8 | 17.9 | 32.2 | 14.0 | 18.8 |
| | UA | 7.39 | 3.5 | 17.9 | 19.8 | 11.0 | |
| 3 | RA | 7.49 | 19.7 | 20.2 | 97.7 | ---- | |
| | IVS | 7.46 | 14.9 | 20.2 | 73.6 | 39.0 | |
| | UV | 7.41 | 11.5 | 23.3 | 49.1 | 22.0 | 21.2 |
| | UA | 7.39 | 6.4 | 23.2 | 27.6 | 13.5 | |
| 4 | RA | 7.44 | 14.8 | 15.7 | 94.3 | ---- | |
| | IVS | 7.40 | 11.8 | 15.7 | 75.1 | 41.0 | |
| | UV | 7.38 | 16.1 | 22.7 | 70.8 | 30.0 | 17.5 |
| | UA | 7.37 | 7.3 | 22.7 | 32.1 | 17.0 | |
| Average | RA | 7.45 | 16.1 | 16.8 | 95.2 | ---- | |
| | IVS | 7.43 | 12.6 | 16.8 | 75.0 | 42.9 | |
| | UV | 7.41 | 11.4 | 20.7 | 54.1 | 22.5 | 24.7 |
| | UA | 7.39 | 5.6 | 20.7 | 26.6 | 13.9 | |

*Key: RA = radial artery, IVS = intervillous space, UV = umbilical vein, UA = umbilical artery.

space value was 7.0 volumes per cent. The difference between the radial artery and intervillous space oxygen content was 9.1 volumes per cent. The mean umbilical vein oxygen content was 8.1 volumes per cent and the umbilical artery 2.5 volumes per cent. These latter two values are considerably reduced over the normal group. However, the arteriovenous oxygen difference across the fetus was approximately the same, 5.6 volumes per cent. In 4 patients, the radial artery oxygen saturation averaged 86.6 per cent (approximately 50.0 mm. Hg oxygen pressure), an average value lower than that found in healthy individuals, namely 95.2 per cent saturation (approximately 80 mm. Hg oxygen pressure). The intervillous space blood was more unsaturated in abnormal pregnancy and averaged 39.9 per cent; oxygen pressure, 26.7 mm. Hg (normal 75 per cent, 42.9 mm. Hg). Umbilical vein and artery values were approximately the same as those seen in the uncomplicated series. The gradient of oxygen pressure between mother and fetus for the abnormal group averaged 12.9 mm. Hg, approximately half the value found in the normal pregnancy group (24.7 mm. Hg) (Fig. 1).

**Carbon dioxide data, uncomplicated pregnancy.** The measured and calculated values for carbon dioxide and standard bicarbonate (bicarbonate measured at a $CO_2$ pressure of 40 mm. Hg, 37° C., with blood fully oxygenated) are presented in Table V. The average for maternal and fetal pH values varied between 7.39 and 7.45, the maternal being the higher in all cases. Under steady state conditions at this altitude, the radial artery and alveolar $CO_2$ pressure in normal pregnant women approximate 29 mm. Hg.[8] The average alveolar $CO_2$ pressure of 26.0 mm. Hg in the normal group probably represents hyperventilation in 2 out of the 4 uncomplicated cases. The carbon dioxide tension gradient between mother and fetus averaged 7.3 mm. Hg. These data agree

**Table IV.** Individual and average determinations of maternal and fetal oxygen in patients who had complications of pregnancy

| *Case No.* | *Vessel** | *pH at 37° C.* | *$O_2$ content (vol. %)* | *$O_2$ capacity (vol. %)* | *$O_2$ saturation (%)* | *Estimated $O_2$ partial pressure (mm. Hg)* | *Estimated $O_2$ pressure difference between mother and fetus (gradient) (mm. Hg)* |
|---|---|---|---|---|---|---|---|
| 5 | RA | 7.48 | 16.7 | 21.1 | 79.0 | ---- | |
| | IVS | 7.38 | 7.1 | 21.1 | 36.3 | 21.0 | |
| | UV | 7.28 | 5.6 | 24.7 | 22.0 | 13.0 | 9.5 |
| | UA | 7.22 | 2.2 | 24.7 | 8.9 | 10.0 | |
| 6 | RA | 7.41 | 14.7 | 16.1 | 91.1 | ---- | |
| | IVS | 7.41 | 12.2 | 16.1 | 76.2 | 40.0 | |
| | UV | 7.27 | 8.6 | 19.4 | 44.5 | 22.0 | 23.0 |
| | UA | 7.19 | 1.8 | 19.4 | 9.3 | 12.0 | |
| 7 | RA | 7.40 | 17.6 | 21.0 | 83.7 | ---- | |
| | IVS-B | 7.20 | 2.0 | 21.0 | 9.2 | 15.0 | |
| | UV-B | 7.20 | 2.6 | 20.5 | 12.4 | 14.0 | 5.0 |
| | UA-B | 7.12 | 0.7 | 20.5 | 3.4 | 6.0 | |
| 8 | RA | 7.35 | 15.3 | 16.5 | 92.5 | ---- | |
| | IVS | 7.36 | 13.3 | 16.5 | 81.0 | 45.0 | |
| | UV-A | 7.19 | 12.2 | 23.3 | 52.2 | 28.0 | 22.0 |
| | UA-A | 7.16 | 5.5 | 23.3 | 23.4 | 18.0 | |
| | UV-B | 7.19 | 13.3 | 20.7 | 64.4 | 33.0 | 19.0 |
| | UA-B | 7.13 | 5.7 | 20.7 | 27.4 | 19.0 | |
| 9 | IVS | 7.26 | 4.8 | 21.3 | 22.3 | 18.0 | |
| | UV | 7.28 | 6.4 | 22.6 | 28.4 | 16.0 | 6.5 |
| | UA | 7.28 | 1.4 | 22.6 | 6.3 | 7.0 | |
| 10 | IVS-A | 7.18 | 5.3 | 17.9 | 29.6 | 26.0 | |
| | UV-A | 7.12 | 9.5 | 22.9 | 41.0 | 26.0 | 9.0 |
| | UA-A | 7.10 | 1.9 | 22.9 | 8.3 | 8.0 | |
| | IVS-B | 7.20 | 4.4 | 17.9 | 24.6 | 22.0 | |
| | UV-B | 7.19 | 6.6 | 20.7 | 32.0 | 20.0 | 9.0 |
| | UA-B | 7.17 | 0.9 | 20.7 | 4.3 | 6.0 | |
| Average | RA | 7.41 | 16.1 | 18.7 | 86.6 | ---- | |
| | IVS | 7.28 | 7.0 | 18.8 | 39.9 | 26.7 | |
| | UV | 7.21 | 8.1 | 21.8 | 37.1 | 21.5 | 12.9 |
| | UA | 7.17 | 2.5 | 21.8 | 11.4 | 10.8 | |

*Key: RA = radial artery, IVS = intervillous space, UV = umbilical vein, UA = umbilical artery, B = second twin, A = first twin.

with those previously reported.[9] The standard bicarbonate was the same in both mother and fetus, approximately 20.0 mM. per liter.

**Carbon dioxide data, complicated pregnancy.** The carbon dioxide data for abnormal pregnancy are presented in Table VI. The arterial pH of the mother averaged 7.41 and ranged from 7.35 to 7.48. The mean pH in the intervillous space was 7.28 while that in the umbilical vein was 7.21 and in the umbilical artery, 7.17 (Fig. 2). These values indicate a maternal acidosis at the level of the intervillous space and a fetal acidosis. The tension or concentration of carbon dioxide in the maternal radial artery averaged 28.8 mm. Hg, the intervillous space, 44.7 mm. Hg, the umbilical vein, 52.0 mm. Hg, and the umbilical artery, 62.2 mm. Hg. These data indicate a respiratory (increase in $CO_2$ concentration) type of fetal acidosis. The average carbon dioxide concentration difference between mother and fetus was 13.5 mm. Hg, or approximately twice the average carbon dioxide gradient found in the parturients with no complications (7.3 mm. Hg) (Fig. 3). The standard

bicarbonate of 18.8 mM. per liter in the mother was slightly below normal, whereas the 15.4 value for standard bicarbonate in the fetus fell considerably below the normal —a finding which indicates that these fetuses in utero also had a metabolic (decrease in $HCO_3^-$) type of acidosis (Fig. 4).

**Hyaline membrane disease data.** The idiopathic respiratory distress syndrome described as a clinical entity in the literature[10] is considered in this study to be identical with hyaline membrane disease. On this basis, the incidence of hyaline membrane disease of the newborn infant in mothers with complicated pregnancy was 77.8 per cent (Table VII). This value represents 7 infants from 9 births. These figures indicate that in a large majority of those infants who developed hyaline membrane disease and who were delivered from mothers with complications of pregnancy, the following disturbances in maternal-fetal relationships occurred: reduction in maternal arterial oxygen saturation, decrease in oxygen pressure gradient between mother and fetus by approximately 50 per cent, increase in carbon dioxide tension gradient to a similar degree, and fetal respiratory and metabolic acidosis (or acidosis caused by an increase in $CO_2$ concentration and a decrease $HCO_3^-$ concentration).

## Comment

It is possible to obtain simultaneous samples of blood which are representative of the maternal-fetal equilibrium. In the present study, success was achieved in the 4 patients who were considered not to have complications and the 6 who did. Although the measurements are not strictly representative of conditions in utero, they are sufficiently so to permit a comparison between the so-called "steady state" (dynamic equilibrium) and the disturbed environment. With the technique standardized in the two groups of patients, the biochemical differ-

**Table V.** Acid-base measurements in the mother and fetus in uncomplicated deliveries

| *Case No.* | *Vessel** | *pH at 37° C.* | *Plasma $CO_2$ (mM./L.)* | *Plasma bicarbonate (mM./L.)* | *Calculated partial pressure $CO_2$ (mm. Hg)* | *Physically dissolved $CO_2$ (mM./L.)* | *Standard bicarbonate $pCO_2$-40 (mM./L.)* | *Calculated $CO_2$ pressure difference between mother and fetus (gradient) (mm. Hg)* |
|---|---|---|---|---|---|---|---|---|
| 1 | RA | 7.44 | 19.8 | 18.9 | 28.7 | 0.86 | 20.3 | |
| | IVS | 7.43 | 19.5 | 18.6 | 29.0 | 0.87 | | |
| | UV | 7.42 | 21.6 | 20.6 | 33.0 | 0.98 | 19.4 | 5.5 |
| | UA | 7.41 | 23.7 | 22.5 | 36.0 | 1.15 | | |
| 2 | RA | 7.43 | 19.2 | 18.4 | 28.6 | 0.86 | 19.8 | |
| | IVS | 7.44 | 20.5 | 19.6 | 30.0 | 0.90 | | |
| | UV | 7.44 | 20.9 | 20.0 | 31.0 | 0.93 | 19.7 | 4.7 |
| | UA | 7.39 | 23.6 | 22.5 | 38.3 | 1.15 | | |
| 3 | RA | 7.49 | 16.6 | 16.0 | 21.7 | 0.65 | 19.8 | |
| | IVS | 7.46 | 17.9 | 17.2 | 24.9 | 0.75 | | |
| | UV | 7.41 | 22.1 | 21.1 | 34.4 | 1.03 | 20.5 | 11.7 |
| | UA | 7.39 | 23.9 | 22.8 | 38.8 | 1.16 | | |
| 4 | RA | 7.44 | 17.0 | 16.2 | 24.7 | 0.74 | 19.3 | |
| | IVS | 7.40 | 19.5 | 18.6 | 30.9 | 0.93 | | |
| | UV | 7.38 | 20.6 | 19.6 | 34.2 | 1.03 | 19.7 | 7.4 |
| | UA | 7.37 | 24.4 | 23.1 | 42.3 | 1.27 | | |
| Average | RA | 7.45 | 18.2 | 17.4 | 26.0 | 0.78 | 19.8 | |
| | IVS | 7.43 | 19.3 | 18.5 | 28.7 | 0.86 | | |
| | UV | 7.41 | 21.3 | 20.3 | 33.1 | 0.99 | 19.8 | 7.3 |
| | UA | 7.39 | 23.9 | 22.7 | 38.8 | 1.18 | | |

*Key: RA = radial artery, IVS = intervillous space, UV = umbilical vein, UA = umbilical artery.

**Table VI.** Acid-base measurements in the mother and fetus in complicated deliveries

| *Case No.* | *Vessel** | *pH at 37° C.* | *Plasma $CO_2$ (mM./L.)* | *Plasma bicarbonate (mM./L.)* | *Calculated partial pressure $CO_2$ (mm. Hg)* | *Physically dissolved $CO_2$ (mM./L.)* | *Standard bicarbonate $pCO_2$-40 (mM./L.)* | *Calculated $CO_2$ pressure difference between mother and fetus (gradient) (mm. Hg)* |
|---|---|---|---|---|---|---|---|---|
| 5 | RA | 7.48 | 18.3 | 17.5 | 24.3 | 0.73 | 17.0 | |
| | IVS | 7.38 | 18.9 | 17.9 | 31.3 | 0.94 | | |
| | UV | 7.28 | 21.8 | 20.4 | 44.9 | 1.35 | 16.3 | 18.9 |
| | UA | 7.22 | 23.6 | 21.9 | 55.5 | 1.67 | | |
| 6 | RA | 7.41 | 18.6 | 17.7 | 28.9 | 0.87 | 20.3 | |
| | IVS | 7.41 | 18.9 | 18.1 | 29.5 | 0.89 | | |
| | UV | 7.27 | 22.0 | 20.6 | 46.5 | 1.40 | 18.1 | 24.5 |
| | UA | 7.19 | 24.6 | 22.7 | 61.5 | 1.85 | | |
| 7 | RA | 7.40 | 17.8 | 16.9 | 28.2 | 0.85 | 18.9 | |
| | IVS-B | 7.20 | 21.2 | 19.6 | 52.0 | 1.56 | | |
| | UV-B | 7.20 | 21.1 | 19.5 | 52.0 | 1.56 | 12.3 | 6.5 |
| | UA-B | 7.12 | 22.5 | 20.2 | 65.0 | 2.27 | | |
| 8 | RA | 7.35 | 19.2 | 18.1 | 33.9 | 1.02 | 19.0 | |
| | IVS | 7.36 | 20.8 | 19.6 | 35.8 | 1.07 | | |
| | UV-A | 7.19 | 20.7 | 19.2 | 51.9 | 1.56 | 15.5 | 20.7 |
| | UA-A | 7.16 | 22.9 | 21.1 | 61.1 | 1.83 | | |
| | UV-B | 7.19 | 19.6 | 18.1 | 49.1 | 1.47 | 15.1 | 20.4 |
| | UA-B | 7.13 | 22.3 | 20.4 | 63.3 | 1.90 | | |
| 9 | IVS | 7.26 | 26.4 | 24.7 | 56.0 | 1.68 | | |
| | UV | 7.28 | 27.2 | 25.6 | 56.0 | 1.68 | | 1.9 |
| | UA | 7.28 | 29.2 | 27.4 | 60.2 | 1.81 | | |
| 10 | IVS-A | 7.18 | 22.1 | 20.4 | 56.6 | 1.69 | | |
| | UV-A | 7.12 | 21.5 | 19.7 | 62.3 | 1.88 | | 10.1 |
| | UA-A | 7.10 | 23.8 | 21.7 | 71.0 | 2.13 | | |
| | IVS-B | 7.20 | 21.1 | 19.6 | 51.8 | 1.55 | | |
| | UV-B | 7.19 | 21.1 | 19.6 | 53.0 | 1.59 | | 4.7 |
| | UA-B | 7.17 | 23.6 | 21.8 | 60.0 | 1.80 | | |
| Average | RA | 7.41 | 18.5 | 17.6 | 28.8 | 0.86 | 18.8 | |
| | IVS | 7.28 | 21.3 | 20.0 | 44.7 | 1.34 | | |
| | UV | 7.21 | 21.9 | 20.3 | 52.0 | 1.56 | 15.4 | 13.5 |
| | UA | 7.17 | 24.1 | 22.1 | 62.2 | 1.91 | | |

*Key: RA = radial artery, IVS = intervillous space, UV = umbilical vein, UA = umbilical artery, B = second twin, A = first twin.

**Table VII.** Correlation of hyaline membrane disease with the average oxygen and carbon dioxide pressure gradients and with average acid-base measurements in mother and fetus

| *Type of pregnancy* | *Incidence hyaline membrane disease (%)* | *Maternal arterial $O_2$ saturation (%)* | *Estimated maternal-fetal $pO_2$ gradient (mm. Hg)* | *Calculated maternal-fetal $pCO_2$ gradient (mm. Hg)* | *Umbilical vein* | | |
|---|---|---|---|---|---|---|---|
| | | | | | *pH (37° C.)* | *$pCO_2$ (mm. Hg)* | *$BHCO_3$ (mM./L.) (standard)* |
| Normal | 0 | 95.2 | 24.7 | 7.3 | 7.41 | 33.1 | 19.8 |
| Complicated | 77.8 | 86.6 | 12.8 | 13.5 | 7.21 | 52.0 | 15.4 |

ences found are due to the disturbed physiologic conditions. It appears from these studies that a parturient without complications is, comparatively speaking, well oxygenated and not in acidosis. Likewise, the fetus in utero, at the time of delivery, is well oxygenated and does not suffer from a respiratory or metabolic acidosis. In contrast, patients with certain types of maternal disease and/or obstetric complications will frequently exhibit one or several of the following biochemical alterations: reduced maternal arterial oxygen saturation, decreased intervillous space oxygen tension, fetal respiratory acidosis, fetal metabolic acidosis, and decreased oxygen pressure gradient across the placenta and/or increased placental carbon dioxide tension gradient. In this study, infants born in such a disturbed environment frequently develop hyaline membrane disease. This finding suggests that a reduced maternal-fetal oxygen pressure gradient and the presence of fetal acidosis which exists in utero might well predispose the newborn infant to pulmonary hyaline membrane disease. Such a concept would be compatible with generalized capillary injury in utero and subsequent localized pulmonary capillary damage at the onset of respiration. Knowledge of capillary permeability, based upon experiments measuring the accumulation of edema fluid, indicates that increased acidity will produce increased capillary permeability.[11] Moreover, oxygen lack will also increase permeability while a return to adequate oxygenation will reverse capillary permeability.[12] Therefore, it appears reasonable to suggest that a persistence after birth of the fetal acidosis which began in utero (with or without oxygen lack) would favor the leakage of nonformed blood elements into the alveoli. Extravascular clotting takes place in the alveoli and fibrin is precipitated as the major component of hyaline membranes.[13-16] The absence of fibrinolysin and the presence of an inhibitor has been demonstrated in the lungs of newborn infants dying of hyaline membrane disease.[17] The occurrence of fetal acidosis in utero may sustain the activity of

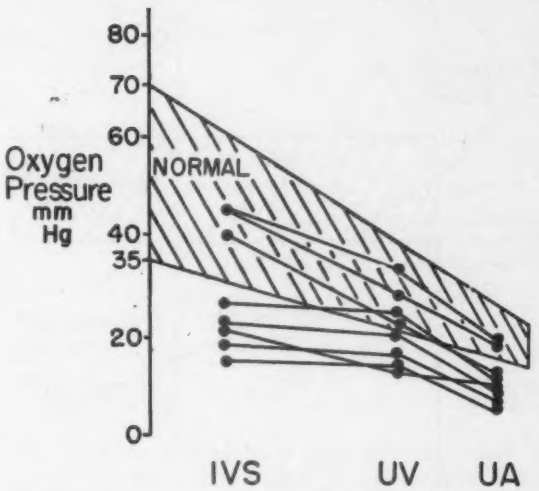


**Fig. 1.** *Comparison of oxygen partial pressures between mother and fetus in uncomplicated and complicated deliveries.* The shaded area is the normal range for oxygen pressure in the intervillous space, umbilical vein, and umbilical artery in 4 uncomplicated patients at term. The solid dots and lines represent the individual oxygen pressures in the same vessels in the 6 complicated cases. The average intervillous space oxygen partial pressure in the complicated group was 26.7 mm. Hg, the umbilical vein being 21.5 mm. Hg and the umbilical artery 10.8 mm. Hg. The difference between these average values in mother and fetus was 12.9 mm. Hg. This was half the oxygen pressure gradient found in the uncomplicated group (24.7 mm. Hg).

the fibrinolytic enzyme system thereby favoring its depletion. This latter suggestion receives support from studies of patients on the pump oxygenator in which increased fibrinolytic activity and the appearance of a circulating anticoagulant was enhanced by poor perfusion and a low pH of the blood.[18] Miller's hypothesis[19, 20] that hyaline-like membrane disease begins as an injurious process in utero is substantiated by the biochemical findings in this study. In turn, these data do not lend support to the concept that amniotic fluid, which bathes the alveoli continuously during pregnancy, suddenly becomes a noxious agent, and is responsible for hyaline membrane disease.[21]

The average duration of pregnancy for the uncomplicated mothers was 39 weeks, while the birth weight of their infants averaged 2,750 grams. One infant was premature by weight (2,450 grams). The duration of pregnancy in the complicated group aver-

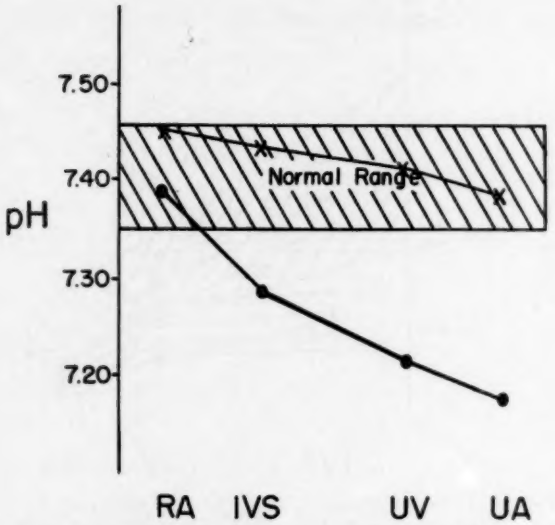


**Fig. 2.** ***Average pH values in mother and fetus in uncomplicated and complicated deliveries.*** The shaded area represents the physiologic range of pH from 7.35 to 7.45 as reported in the literature. The cross marks within this area represent the pH of the mother without complications (radial artery and intervillous space) and the fetus (umbilical vein and umbilical artery) in utero. These average values range from 7.45 to 7.39 pH units. In contrast, mothers with complicated pregnancies (solid dots and lines) show low pH values at the level of the intervillous space, umbilical artery and umbilical vein.

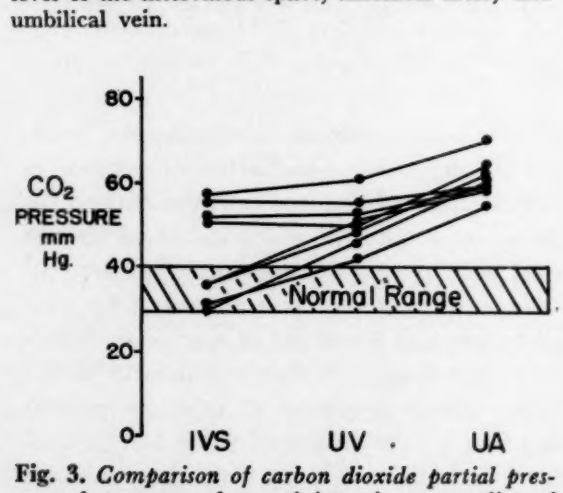


**Fig. 3.** ***Comparison of carbon dioxide partial pressures between mother and fetus in uncomplicated and complicated deliveries.*** The shaded area represents the carbon dioxide tension in the intervillous space, umbilical vein, and umbilical artery in uncomplicated term pregnancy. In contrast, the solid dots and lines represent the individual carbon dioxide tensions in the same vessels in complicated pregnancy. These average values were 44.7 mm. Hg carbon dioxide tension in the intervillous space, 52.0 mm. Hg in the umbilical vein and 62.2 mm. Hg in the umbilical artery. The difference between these values in mother and fetus was 13.5 mm. Hg. This value is twice the normal carbon dioxide tension gradient found in the uncomplicated group (7.3 mm. Hg).

aged 36 weeks with an average infant birth weight of 2,500 grams. Further evaluation of the 9 infants born to the women who had complications of pregnancy reveals that 3 were term and 6 were premature by weight. Two of the term infants appeared well and healthy at birth. One of the term infants developed hyaline membrane disease and survived, while all of the premature infants had some form of respiratory distress in the immediate neonatal period. One of the premature infants died and at autopsy the lungs showed well-developed hyaline membranes. In the remaining 5, a clinical diagnosis of hyaline membrane disease was made on the basis of chest retraction, expiratory grunting, tachypnea, and cyanosis, which occurred shortly after birth and persisted for 12 to 48 hours. The biochemical alterations found in these mothers and infants might be associated with the cause of premature labor and/or with the stage of gestation per se. Those explanations however, do not appear to be valid because intervillous space oxygen measurements made during the latter part of pregnancy reveal no lowering of oxygen saturation until the thirty-sixth week of gestation.[22] More significant is the fact that other unpublished studies from this laboratory indicate that in uncomplicated premature delivery, fetal acidosis is not present in utero. That intrauterine acidosis cannot be the only factor in the development of hyaline membranes is suggested by the clinical fact that some premature infants do experience hyaline membrane disease after an uncomplicated delivery.

Measurements of maternal-fetal oxygenation and acid-base balance have been carried out by Barcroft,[23] Barron and associates,[24-28] Eastman and associates,[29, 30] Prystowsky and associates,[1, 31] and Kaiser,[32, 33] as well as others. Many of these investigators have been careful to point out that measurements of oxygen saturation or tension are not necessarily indicative of hypoxia. The present studies, therefore, do not prove that an infant born to a mother with a disturbed pregnancy suffers hypoxia in utero. These data, however, do demonstrate that infants

born of mothers whose arterial and intervillous space oxygen pressures were lower than normal, did have respiratory and metabolic acidosis and subsequently developed hyaline membrane disease in 7 out of 9 cases. Furthermore, no such incidence occurred in the so-called normal biochemical environment.

Clinically speaking, it seems reasonable to suggest, on the basis of other studies[34, 35] and unpublished data from this laboratory, that prophylactic oxygen be administered to patients during labor and delivery. This is especially important at the time of elective or indicated cesarean section when maternal oxygen saturation may be transiently reduced by analgesia, anesthesia, positioning, and the enlarged pregnant uterus. Present evidence indicates that in normal pulmonary circulation mechanical influences (intra-alveolar pressure, intrapleural pressure, pulmonary blood flow, and pulmonary blood volume) are of prime importance when compared to vasomotor factors.[36] The normal subject in the supine position regulates pulmonary ventilation and circulation quite well. The pregnant patient, especially an obese individual, placed on the side, given a spinal anesthetic, and then turned to the supine position may be an excellent candidate for profound circulatory and ventilatory disturbances. This may be the explanation for the reduction in arterial oxygen saturation in 4 of the 6 patients with complications of pregnancy since none of these mothers had primary cardiac or pulmonary disease.

Preliminary experiments in this laboratory indicate that an amine buffer, trihydroxymethylaminomethane (THAM), will prevent respiratory and metabolic acidosis in both maternal and fetal rabbits made acidotic by "diffusion respiration."[37-39] In time, this may have clinical significance in patients who have these complications of pregnancy.

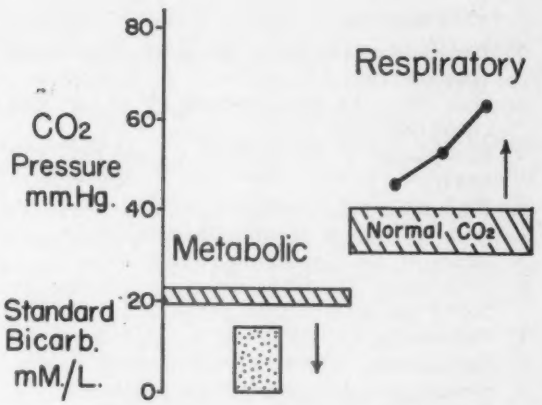


**Fig. 4.** *Type of acidosis present in infants born to mothers with complications of pregnancy.* This illustration shows that the components of fetal acidosis are a decrease in bicarbonate concentration and an increase in carbon dioxide concentration. The normal standard bicarbonate is represented by the narrow shaded area (standard bicarbonate refers to bicarbonate measured at a standard $pCO_2$ of 40 mm. Hg, 37° C., with the blood hemoglobin fully oxygenated. This was done to eliminate the respiratory [$CO_2$] aspect of acidosis). A reduction in fetal standard bicarbonate to an average of 15.4 mM. per liter represents metabolic acidosis. The normal range for $CO_2$ pressure in the fetus is shown by the larger cross-hatched area. The solid dots and lines are the average carbon dioxide pressures in the intervillous space, umbilical vein, and umbilical artery. This increase in fetal carbon dioxide pressure or $H_2CO_3$ may be spoken of as respiratory acidosis.

## Summary and conclusions

1. A pregnant mother without complications and the fetus in utero are apparently sufficiently well oxygenated and in an undisturbed acid-base equilibrium at the moment of delivery.

2. Gravidas with certain complications of pregnancy and/or delivery show values that are lower for the maternal arterial and intervillous blood oxygen saturation at delivery.

3. In mothers with certain complications of pregnancy the fetus in utero has a measurable amount of respiratory and metabolic acidosis.

4. It is postulated that hyaline membrane disease in the newborn infant, which is known also as the "idiopathic respiratory distress syndrome," may begin as a biochemical injury to the fetus in utero manifested by respiratory and metabolic acidosis and a reduced maternal-fetal oxygen pressure gradient.

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## Discussion

Dr. Charles E. McLennan, Palo Alto, California. Dr. Bruns has presented a topic of current interest to pediatricians and one that perhaps should be of increasing interest to obstetricians, particularly if it can be shown that we have a role in the prevention of idiopathic respiratory distress syndrome of the newborn infant. However, if anyone believes there is general agreement among pediatricians as to the precise definition of this syndrome, he should read in *The Journal of Pediatrics* (vol. 57, p. 905, 1960) what is termed "An International Exploration" of the problem, representing a verbatim report of a symposium held during the International Congress of Pediatrics in Montreal. There were 34 participants, and their views on many facets of the subject were at that time amazingly discordant.

In the present study, the oxygen data show so much variation that one really cannot draw any firm conclusions from them. Dr. Bruns clearly points out that oxygen consumption cannot be determined from $pO_2$ alone, and certainly the maternal-fetal gradient does not tell anything about the *rate* of diffusion. I believe that the electrolyte data are far more consistent and warrant the conclusion that the fetuses in question did have a respiratory acidosis complicated by a metabolic acidosis. But Dr. Bruns goes further and says that 7 of 9 acidotic infants developed hyaline membrane disease, although only one of these died and permitted demonstration of the typical membranes. I think it might be more realistic to say that 6 mothers who were delivered prematurely were studied and 4 of them (2 with toxemic twin pregnancies, 1 with diabetes, and 1

with twins) produced 7 infants showing the clinical picture of respiratory distress. One wonders, of course, whether this 2 out of 3 coincidence of abnormal pregnancy and fetal distress is a bizarre statistical sample or whether it represents a real association of phenomena. No data from control cases are given, although Dr. Bruns alludes to unpublished studies showing that fetal acidosis is not found in premature infants of ostensibly normal mothers. One may ask why these data were not presented to strengthen the argument.

Speaking of statistics, I was distressed to note the following—"the incidence of hyaline membrane disease of the newborn infant in mothers with complicated pregnancy was 77.8 per cent (Table VII)." Assuming the role of amateur statistician, I feel I must decry the use of percentages to describe samples of half a dozen, and most particularly the inclusion of a digit to the right of the decimal point. Percentages of this sort tend to be remembered by readers with photographic minds and ultimately find their way into the folklore of staff conferences (without reference to the basic data). A recent pediatric source gives 14 per cent as the incidence of respiratory distress syndrome in all liveborn premature infants. I find it hard to accept the notion that nearly 80 per cent of premature infants born to women with pre-eclampsia and/or diabetes may turn up with this syndrome; possibly Dr. Bruns did not intend to convey this impression.

The finding of low arterial $pO_2$ in women with complicated pregnancies was quite striking and perhaps surprising. The essayist has speculated about the combination of obesity and spinal anesthesia leading to circulatory and ventilatory disturbances, but I wonder if he would care to develop this thesis further and say whether there were, indeed, obvious difficulties at the head end of the table in all of these instances.

I wonder, too, whether Dr. Bruns would wish to comment on the work done in Montreal by Usher, who found acidosis and elevated plasma potassium concentrations in premature infants who had breathing difficulties. Presumably Usher's treatment of all such infants with intravenous glucose and sodium bicarbonate has lowered the neonatal death rate quite remarkably. Should we perhaps advocate this regimen in addition to the prophylactic use of oxygen during labor and delivery?

The work of Jost with fetal animals indicates, but has not proved, that amniotic fluid is to a great extent produced by fetal lung. Is it possible that analyses of amniotic fluid from mothers with abnormal pregnancies might be a worthwhile venture? I note that Dr. Bruns has rejected Snyder's suggestion that amniotic fluid can become a noxious agent, but in a recent paper Rooth and Sjöstedt (Am. J. Obst. & Gynec. 81: 4, 1961) claim that a proper interpretation of the acid-base balance of the amniotic fluid may give important information about the fetus. These workers claim, for instance, that measurements of $pCO_2$ in amniotic fluid are an indication of intrauterine oxygenation of the fetus. They point out further that in late pregnancy high $pCO_2$ values are paired with low pH values, indicating that the fetal kidneys are trying to regulate pH by increasing their excretion of acid, thus establishing a metabolic acidosis in the amniotic fluid. Is this a factor in hyaline membrane production or is it merely the end result of dysfunction in some other physicochemical mechanism?

# The origin and distribution of oxytocinase

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ONE of the enigmas in the field of obstetrics is the appearance in maternal plasma of a new enzyme which is capable of inactivating oxytocin or vasopressin with considerable rapidity. It is present only in the blood of pregnant primates, and its activity increases progressively until the last month of gestation. The ability of pregnancy plasma to destroy oxytocin (Pitocin, Syntocinon) was first noted by Fekete[1] in 1930 and has since been studied by many investigators. To date, however, the origin and distribution of the enzyme are poorly understood and its function in the body is completely unknown.

Progress in this area was stimulated when duVigneaud and associates[2] and Tuppy[3] independently in 1953 described the sequence of amino acids in the oxytocin molecule, knowledge which led within a few months to its total synthesis. In 1957, Tuppy and Nesvadba[4] demonstrated that the action of pregnancy plasma was to split tyrosine from that half of cystine bearing the terminal amino group, thus opening the pentapeptide ring portion of oxytocin to render it inactive. Oxytocinase, therefore, is a cystine aminopeptidase (CAP), and its activity may be measured chemically by the use of a synthetic substrate, cystine di-beta naphthylamide.[5] The change of CAP activity is progressively upward as pregnancy advances and does not decrease just before or during labor.[6] Hydrolysis of cystine di-beta naphthylamide by CAP in nonpregnancy serum is measurable, whereas oxytocinase activity as measured by the rate of inactivation of oxytocin and the use of a rat uterus bioassay is negligible.[7, 8] This may be because the synthetic naphthylamide substrate is split slightly by some aminopeptidase in nonpregnancy serum which does not affect oxytocin.

In this brief communication, we wish to demonstrate that there are two species of cystine aminopeptidases in human pregnancy plasma; that neither can be found in fetal blood, but both may be found in maternal urine; and that the syncytiotrophoblast is the most probable cell of origin for both of the new enzymes.

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## Methods

The vertical starch gel electrophoresis technique of Smithies[9] was used for the separation of the various proteins in serum, urine, or tissue extracts. The electrophoresis was carried out in the refrigerator at a temperature of about 5° C. for 17 hours. With use of 260 volts, a current of 20 to 25 milliamperes was produced under these conditions.

When the enzyme bands were to be eluted, a gel of 1.9 cm. thickness was prepared as suggested by Moretti[10] and run at 200 volts

with a current of 43 to 50 milliamperes. A serum sample of 2.0 to 2.5 ml. could then be introduced into a trench extending along the full width of the gel. The bands were located from a stained slice, then cut out and digested in 2 ml. of 5 per cent diastase solution for 4 hours at 37° C. After centrifugation, the supernatant solutions were used for analysis of CAP activity with or without oxytocin inhibition.

After the gels were sliced with the aid of a fine steel wire, one half was stained for proteins with amido black 10B and the other half was stained histochemically for leucine aminopeptidases by means of the Gomori technique as described by Burstone and Folk.[11] More recently we have used Black K salt, as described by Lawrence, Melnick, and Weimer,[12] in place of the fast garnet GBC salt. The former is superior when photographic records of gels are to be made.

Urinary proteins were concentrated by placing a 24 hour urine specimen in dialyzing tubing surrounded by solid polyvinyl pyrrolidone which extracts water and dialyzable salts. The volumes were concentrated 17- to 100-fold depending on the protein content of the urine sample.

## Results

With our electrophoresis technique, we always find two new bands in pregnancy serum. Their location, compared to the protein pattern, is shown in Fig. 1 and is further summarized in Table I. For the sake of convenience, we shall refer to these enzyme bands as LAP, which appears in all human sera; $CAP_1$, the enzyme which closely trails LAP and which has apparently not been observed before; and $CAP_2$, the enzyme which trails the $beta_1$ globulin (transferrin). These designations are used because each of the pregnancy enzymes, when eluted from the gel, demonstrates cystine aminopeptidase activity, whereas the LAP band when eluted shows negligible CAP activity. All three have LAP activity, i.e., all three utilize leucyl di-beta naphthylamide as a substrate. Wintersberger and Tuppy[13] described a single new band in pregnancy plasma stained for leucine aminopeptidase (LAP). When plasma was stained with cystine di-beta naphthylamide as a substrate, only this new band appeared, showing that LAP has very little, if any, CAP activity.[14]

We have never been able to demonstrate the CAP enzymes in fetal serum, which is in accord with the absence of biologically active oxytocinase in fetal blood.[7] We have never observed the enzymes in the blood of a nongravid individual. In some patients with liver disease or advanced malignancy, we found several species of LAP. In 2 men and 1 woman with active choriocarcinoma and positive tests for chorionic gonadotropin the CAP bands were absent. This suggests that the cell (the cytotrophoblast) which liberates chorionic gonadotropin does not liberate cystine aminopeptidase (oxytocinase).

Electrophoresis of the protein concentrated from urine collected late in normal pregnancy results in faint LAP and $CAP_2$ bands. In patients with pre-eclampsia all three bands are found. Thus, the plasma aminopeptidases leak through the glomeruli along with albumin, transferrin, and other proteins.

It is not known whether $CAP_1$ and $CAP_2$ differ qualitatively in their preference for endogenous peptide structures for we have not yet characterized them biologically. The eluates from both bands, however, are significantly inhibited in their activity on cystine di-beta naphthylamide when synthetic oxytocin is added. This is presumably due to competitive inhibition and supports

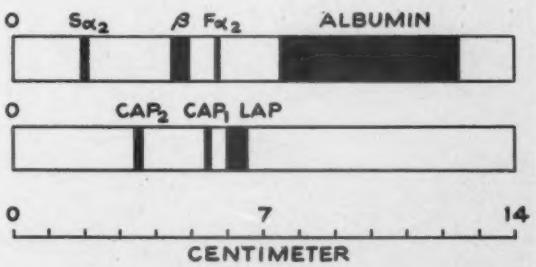


**Fig. 1.** Diagram illustrating the location of the two cystine aminopeptidase bands ($CAP_1$ and $CAP_2$) and leucine aminopeptidase (LAP). Upper strip shows the major serum protein bands as they appear on the starch gel.

**Table I.** Relative positions of bands in non-pregnancy and pregnancy sera and in placental extracts*

| | *No.* | *Albumin* | *LAP* | *$CAP_1$* | *Beta* | *$CAP_2$* | *Slow $A_2$* |
|---|---|---|---|---|---|---|---|
| Nonpregnancy sera | 9 | 100 | 44.5 | — | 32 | — | 16.2 |
| Pregnancy sera | 14 | 100 | 45.5 | 40 | 35 | 28 | 16.5 |
| Placental extracts | 7 | 100 | 44.0 | 39 | — | 28 | — |

*The albumin front is expressed as 100 per cent. All other values are expressed as the per cent of this distance. LAP is leucine aminopeptidase. $CAP_1$ and $CAP_2$ are the two cystine aminopeptidases specific for primate pregnancy.

the thesis that there are two species of oxytocinase in human pregnancy plasma.

The enzyme $CAP_1$ seems to appear first in early pregnancy, although in the third trimester the $CAP_2$ band appears more dense. This is illustrated in Fig. 2, which is a photograph of a gel containing 8 sera from 8 different women at varying periods of pregnancy and stained for LAP activity. Quantitative measurements of CAP activity were also made on aliquots of each serum. Units are expressed in milligrams of naphthylamide released per 100 ml. of plasma per hour. The

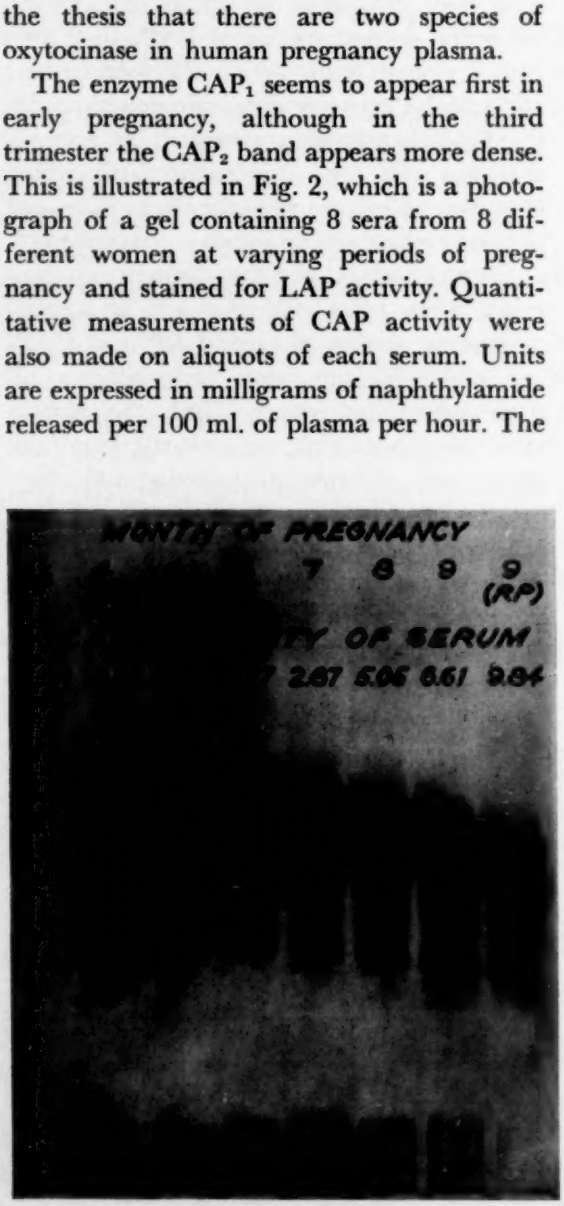


**Fig. 2.** Photograph of a starch gel containing eight sera as noted and stained for leucyl aminopeptidase activity. *RP* indicates retroplacental blood.

month of pregnancy and the quantitative chemical CAP activity are indicated on each strip. The highest value was in serum obtained at term from retroplacental blood. It will be noted that the LAP band does not appear to increase as pregnancy progresses. This suggests that the increase of leucine aminopeptidase activity found in pregnancy plasma by Green,[15] Arst, Manning, and Delp,[16] Siegel,[17] Bressler and Forsyth,[18] and others may simply be a summation of the effects of all three enzymes, each of which is active on leucyl substrates.

It is difficult to demonstrate aminopeptidases in tissue extracts by the starch gel electrophoresis technique. Some of our extracts from human placentas, however, have shown the LAP, $CAP_1$, and $CAP_2$ bands. Their location relative to the plasma bands is given in Table I. We have not found CAP bands corresponding to those of pregnancy sera in extracts of other human tissues (myometrium, ovary, decidua, Fallopian tube). These tissue extracts contain CAP activity when measured quantitatively. When the extracts are subjected to gel electrophoresis, the dark staining of the site of origin shows that much of the aminopeptidase activity is present in a form incapable of moving through the gel. It may be significant that only pregnancy plasma and placental extracts show significant inhibition when concentrates of oxytocin are added.

## Comment

It would seem almost certain that the human placenta is the source of the two cystine aminopeptidases which appear in the

blood of pregnant women. The reasons are these:

1. The enzymes have never been observed in a nonpregnant individual. The rate of disappearance following delivery follows the rate of disappearance of other proteins peculiar to pregnancy, such as chorionic gonadotropin.

2. The plasma concentration roughly parallels the total mass of the placenta. The highest values we have found have been in multiple pregnancies.

3. Only plasma and placental extracts show significant inhibition of CAP activity when oxytocin is added.

4. The absence of CAP in fetal blood eliminates the fetus itself as a source.

5. The specific CAP bands can be demonstrated in some placental extracts.

6. Hooper and Jessup[19] prepared fractions of human placental extracts by differential centrifugation and found that oxytocinase is almost exclusively in the soluble protein fraction whereas vasopressinase is associated predominantly with mitochondria and microsomes.

It would seem likely that the syncytiotrophoblast is the particular cell of origin for the following reasons:

1. The rise of oxytocinase activity in pregnancy blood follows the curve of syncytial bulk as observed histologically rather than the curve of the cytotrophoblastic bulk (Langhans' cells) which diminishes markedly after midpregnancy.

2. The CAP concentration in pregnancy plasma parallels the estrogen concentration in blood during pregnancy rather than the chorionic gonadotropic hormone concentration. It is believed, from histochemical studies, that the syncytium is the source of estrogens, and the cytotrophoblast is the source of gonadotropin.

3. Because the cell of origin must demonstrate histochemical LAP activity, the syncytiotrophoblast should demonstrate intense LAP activity. Dr. James Merrill has prepared sections of the placenta showing intense LAP activity in the syncytium.

4. Dr. K. Semm, of Munich, has developed a histochemical stain using the synthetic cystine substrate and finds the enzyme activity primarily in the syncytium.[20]

Tuppy and Wintersberger[14] have shown that oxytocinase purified from pregnancy plasma will form an antibody in the rabbit. It is possible that $CAP_1$ and $CAP_2$ are the antigens found in normal pregnancy plasma by MacLaren and co-workers.[21]

## Summary

When serum or urinary proteins from pregnant women are subjected to starch gel electrophoresis and then stained for leucine aminopeptidase activity, three distinct bands appear. The fastest-moving protein is leucine aminopeptidase, found in all nonpregnant as well as in all pregnant individuals. The second and third bands, specific for pregnancy in primates, are cystine aminopeptidases. These enzymes have the capability of inactivating oxytocin.

The two cystine aminopeptidases found in the serum and urine of pregnant women cannot be found in fetal blood or in the blood of men or nongravid women, even those who have choriocarcinoma. The source of the new enzymes is almost certainly the placenta and in all likelihood the syncytiotrophoblast. Their precise physiologic role during pregnancy is unknown.

We are indebted to the Sandoz Company for a donation of Syntocinon concentrate used in these studies.

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## Discussion

Dr. Robert A. Ross, Chapel Hill, North Carolina. In 1946 the author presented a paper at this Society's meeting in which he discussed the presence of an enzyme in the blood of pregnant women which destroyed the oxytocic properties of pitocin. This "pitocinase" enzyme was not found in nonpregnant women or in the cord blood of 5 fetuses. The plasma concentration of this protein increased in logarithmic fashion during pregnancy with a rapid decrease after delivery. There was a scattered recording in true eclampsia. It was hypothesized that the trophoblastic syncytial cells contributed this protein to the maternal blood stream. Similar effects with pregnancy serum had been previously reported and it had been demonstrated that such serum also was "antagonistic" to the vasopressor activity of the posterior pituitary extracts.

In 1953 the sequence of amino acids in the oxytocin molecule were described which resulted in its synthesis. In 1957 it was demonstrated that the action of pregnancy plasma was to split the linkage between cystine and tyrosine. Oxytocinase, therefore, is a cystine aminopeptidase and can be measured chemically by using a synthetic substrate.

In recent years this Society has had several presentations which might be considered germane to this topic. Reid and co-workers, by a serologic method, have demonstrated an immunospecific characteristic of normal pregnancy serum which suggests two substances peculiar to this serum. The findings by G. W. Douglas of syncytiotrophoblast in large numbers in maternal blood and the finding of similar cells by Mack in fetal cord blood are provocative studies.

Nearly every biochemical or physiologic alteration in the pregnant woman is attributable to the presence and activity of the placenta. It is definitely an advocate for the fetus. Only in disease or accident does it fail in this endeavor. The syncytiotrophoblast is the working unit of the placental membrane; acquiring, exchanging, altering, blocking, and elaborating substances in the interest of the fetus. We wholeheartedly agree that the syncytiotrophoblast probably produces oxytocinase, though its rise apparently more closely parallels that of estrogen while its rate of disappearance follows that of chorionic gonadotropin. In the patients who had choriocarcinoma, however, the CAP bands were absent. Surely these tumors contained syncytiotrophoblasts.

The authors state that "we have not characterized them [the enzymes] biologically." They, however, have apparently isolated the enzyme on starch gel and have at least characterized them as to electrophoretic mobilities at both a specific buffer composition and a specific pH. It is possible that the two enzymes represent two components of one protein, both components possessing enzymatic activity after the gentle fractionation by starch gel electrophoresis.

The statement is made, "each of the pregnancy enzymes, when eluted from the gel, demonstrates CAP activity." Classically, one would wish to know whether the eluate was a homogenous protein fraction by such techniques as gradient centrifugation or solubility curves: is it inactivated by dialysis? A synthetic cystinyl-aminopeptide is used for assay. Binkley (J. Am. Chem. Soc. 82: 987, 1960) claims that there are

nonprotein cystinylaminopeptidases in many tissues. The inhibition of the eluates from both bands in their CAP activity after synthetic oxytocin is added is "presumably due to competitive inhibition." The other possibility is that the oxytocin is absorbed but not split by this protein. The difference between competitive and absolute inhibition can be distinguished. And, finally, was the work of Tuppy and Winterberger reproduced and was the migration of their oxytocinase related to this fraction?

When the word "oxytocin" is used, it will have an impact on the clinical use of this powerful hormone. We should hope that further work will lead toward the additional clarification of the indwelling checks and counterchecks that nature supplies in its continuous offensive and defensive endeavor. In this instance, the significance of these enzymes and hormones is the maintenance and termination of pregnancy. To me, it is not difficult to reconcile the control and utilization of the most powerful hormone, oxytocin, with the idea of "progesterone block" in this complete operation.

DR. RUSSELL R. DE ALVAREZ, Seattle, Washington. I have had the privilege of reading this fine paper which Dr. Page sent to Dr. Afonso, of our department, who is working in this area of electrophoresis. Dr. Page has found three separate protein bands in pregnant patients which are aminopeptidases, two of which are specific for pregnancy.

Using starch gel electrophoresis in search of confirmation of the findings reported by Smithies and Giblett in *Advances of Protein Chemistry,* September, 1960, we attempted to demonstrate a zone specific for pregnancy. Smithies and Giblett found a zone specific for pregnancy which they thought occurred in 10 per cent of pregnancies and only in the last trimester of pregnancy. The studies in our department have been carried out in a large group of normal pregnant patients, with use of starch gel electrophoresis throughout pregnancy, labor, delivery, and the postpartum period. The pregnancy zone is specific for pregnancy, makes its appearance usually in the first trimester, and disappears after pregnancy. In a few, it does not appear until during labor. The over-all occurrence is 86 per cent of pregnant patients, with the zone appearing in 82 per cent prior to labor while the remainder occur after the initiation of labor.

The one thing which we have not utilized is the Gomori staining method which is specific for aminopeptidase. In the amido black preparation, the pregnancy zone is located in the haptoglobin area of the electropherogram. When stained with benzidine, the haptoglobins are visualized but the pregnancy zone does not stain. In reviewing the articles of Tuppy, to determine whether he has attempted the same thing, we find that he probably has, but we do not feel that he has demonstrated the same pregnancy zone. Whether the pregnancy zone of Smithies is Dr. Page's cystine aminopeptidase No. 2 we do not know, but we are going to embark on multiple staining methods as soon as we take the news and the composition of the stain back to Seattle.

DR. PAGE (Closing). With respect to the possible fractionation of protein by the method of electrophoresis, it is my understanding that this does not occur. A single pure protein should always migrate as a single band.

Oxytocinase is reduced in activity by prolonged dialysis, allegedly because of the loss of a divalent metal ion upon which its activity depends.

We have not purified the enzyme from plasma or studied its antigenicity, as have Tuppy and Wintersberger, but this work is in progress. As Dr. Ross surmised, we are also attempting to characterize the biologic activity of each enzyme.

I am grateful to Dr. de Alvarez for showing his excellent illustrations of the "pregnancy zone." We have not dealt with this protein, and I presume that it might be the same as the slow oxytocinase band. This should be simple to prove or disprove by using three stains on the same gel strips.

# Serum protein fractionation in normal pregnancy

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THE proteins in the blood provide an accessible index of protein metabolism and thus a better understanding of the unique and versatile nature of their function. The total protein concentration of the plasma in normal nonpregnant patients ranges from 6.5 to 7.9 Gm. per 100 ml. The plasma proteins consist of albumin, a relatively small molecule among proteins, while the globulins are larger but more vaguely characterized. The serum proteins are the same as those found in the plasma, except that fibrinogen is found in only minute quantities as contrasted to the large amounts present in plasma.[18] If all the information were known regarding each function of every constituent of the proteins, it would be possible to formulate a complete list of every component present in any amount. Inasmuch as all functions of all constituents are not adequately known, current classifications are devised to supply information in a form which can be handled practically.

Present evidence strongly suggests that the liver is the principal site of protein synthesis, producing all the functions in the blood with the exception of gamma globulin, which originates from the reticuloendothelial system, particularly the plasma cells.[9-11] In addition to hepatic synthesis, alpha and beta globulins also are synthesized in the bone marrow.[12] Each protein fraction possesses its own rate of synthesis. Thus, alterations in the manufacture of the various fractions should suggest alteration of physiologic processes in those organ systems responsible for synthesis of a given fraction. Albumin and gamma globulin are synthesized somewhat more slowly than are the other protein constituents,[12, 13] with the half life of the average plasma protein being approximately 8 days.[14, 15] As with synthesis, protein catabolism takes place largely in the liver.[15] In addition to catabolic processes, proteins are lost by other mechanisms. Normally, a small amount of protein is present in the urine, with a maximum excretion of 100 to 150 mg. per 24 hours.[16, 17] In disease states, however, this may be greatly increased. In the presence of denudation of areas of skin or mucosa, protein is lost in significant amounts.

Early techniques for measuring the blood proteins involved salting out procedures similar to those developed by Howe[1] in the early 1920's. In 1937, electrophoresis was introduced by Tiselius.[2] Proteins migrate in an electric field except at the pH of the isoelectric point, a useful feature in determining the properties of protein mixtures. At the isoelectric point there is no net charge, but, as acid is added, protein acquires an increasing net positive charge until the maximum acid-binding capacity is reached. Similarly, when alkali is added the net negative charge of the protein is increased. The rate of mi-

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gration of a molecule in an electric field is largely dependent on the net charge, so that the rate of electrophoretic migration will depend on pH in the same manner as does the degree of ionization. Since proteins differ in their isoelectric points and in their titration curves, they differ in electrophoretic mobility at any given pH value so that electrophoretic analysis may be used not only to determine the purity of individual proteins but to determine quantitatively the analysis of complex mixtures. Each component of the mixture migrates at a different rate, resulting in separation of the protein fractions into distinct zones.

Several investigators[3-5] working almost simultaneously developed the technique of paper electrophoresis in 1950. The introduction of this technique made available not only a signal advancement in technology but also a clinical tool to replace some rather cumbersome research procedures. Unlike classical electrophoresis, which is restricted for practical purposes to substances with high molecular weight, paper electrophoresis may be applied to the study of substances of high and low molecular weights, except for those proteins of extremely high molecular weight (viruses, hemocyanins) and asymmetric structure (fibrinogen). Paper electrophoresis is particularly applicable to serum analyses of protein composition, and thus permits the study of many clinical problems. The introduction of starch-gel electrophoresis[6-8] offers even greater precision for identification of serum proteins than did previously reported methods. The single drawback of starch-gel electrophoresis is its failure to permit easy quantitation.

Albumin and globulin may be separated roughly by chemical techniques. However, electrophoresis not only permits an even finer separation of these two principal groups but also results in more adequate partitioning of their subgroups. By the process of paper electrophoresis, in addition to the separation of the two major groups, the four major globulins are separated and classified, according to their relative electrophoretic mobility, as alpha-1, alpha-2, beta, and gamma globulin. However, free boundary electrophoresis of plasma permits the identification of phi globulin (fibrinogen).

By means of starch-gel electrophoresis, the major globulin fractions have been separated into many subfractions.[6-8] In the alpha-2 globulin alone, at least 10 different components are recognized, among which are the haptoglobins or hemoglobin-binding proteins which are genetically inherited, constituting 5 specific genotypes.[19-22] The copper-binding protein, ceruloplasmin, also is a subfraction of the alpha-2 globulin.[23] Another subfraction, referred to as the alpha-2 macroglobulin or the slow alpha-2 globulin, is a high molecular weight glycoprotein.[8, 24] Alpha-1 globulin has been found to contain at least 3 subfractions, one of which has been defined as alpha-1 acidic glycoprotein.[25] The beta globulin has at least 4 different components. The iron-binding serum proteins, transferrins, migrate as beta globulin in the filter paper electrophoresis. In addition to the haptoglobins, the transferrins also are genetically controlled and several genotypes have been described.[26-29] Subfractionation of gamma globulin is possible by zone electrophoresis in a starch or polyvinyl supporting medium revealing multiple components of different mean mobilities.[30]

Many functions of these various components are known but there are undoubtedly many unknown ones. Of the known functions, the maintenance of electrolyte and water balance is very important. While all proteins function to some extent in the osmotic maintenance of blood volume, albumin, because of its small size and greater concentration, is the most important.[31, 32] In combination with hemoglobin, the plasma proteins also provide a buffering action which is at least as great as that of the inorganic buffer systems in the blood.[33] By contrast, many of the protein functions are definitely limited to specific fractions—the antibody response, for instance, is largely limited to gamma globulin.[34]

Transport is a well-known and well-established function of the serum proteins. In some instances, the binding is rather loose,

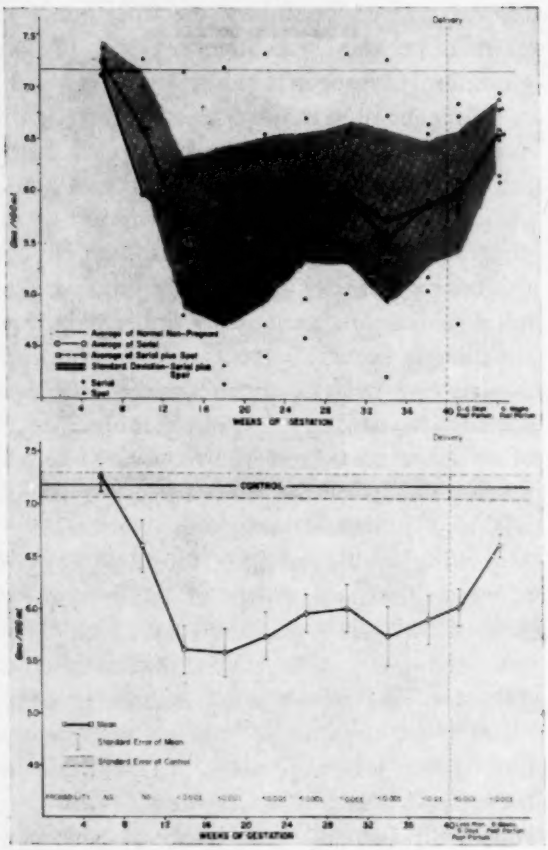


**Fig. 1.** ***Serum total proteins determined during normal pregnancy.*** All determinations are grouped by weeks of gestation ante partum, and into early and late postpartum groups. The control group is composed of normal, nonpregnant, young adult women.

but this depends upon the concentrations of the substance to be bound and upon the concentration of the proteins involved. Corticosteroids and related compounds are bound in this manner with a decrease in the specificity of binding as the concentration of the corticosteroid rises.[35-38] Thyroxin appears to combine specific and nonspecific binding: 80 per cent is localized in and just beyond the alpha-2 globulin area while the remainder is found in all the other protein fractions.[39] Several proteins, such as haptoglobin, transferrin, and transcortin, have been shown to be quite specific in their carrier function.

Blood clotting is a function of many of the plasma constituents including the blood proteins. Fibrinogen is, perhaps, best known in this regard but the alpha and beta globulins also share the role as coagulation and anti-coagulation factors.[40] At least one component in this scheme (heparin cofactor, albumin X) has been found[41] migrating with albumin.

Metabolic interrelationships exist between the plasma proteins and other tissue proteins. In protein deprivation, however, the proteins of the blood are spared at the expense of the structural proteins.[42] Enzymes make up a number of the proteins of the blood, being made available from the body cells. Enzymes enter into practically every physiologic function. In biochemical reactions they permit rapid activity of reaction through the mechanism of catalysis. In addition to their principal role as catalysts, they have assumed great importance in heart disease and pancreatic disease,[43, 44] the best known of which are the various phosphatases, amylase, lipase, and transaminase.[43-46]

With the availability of newer methods to

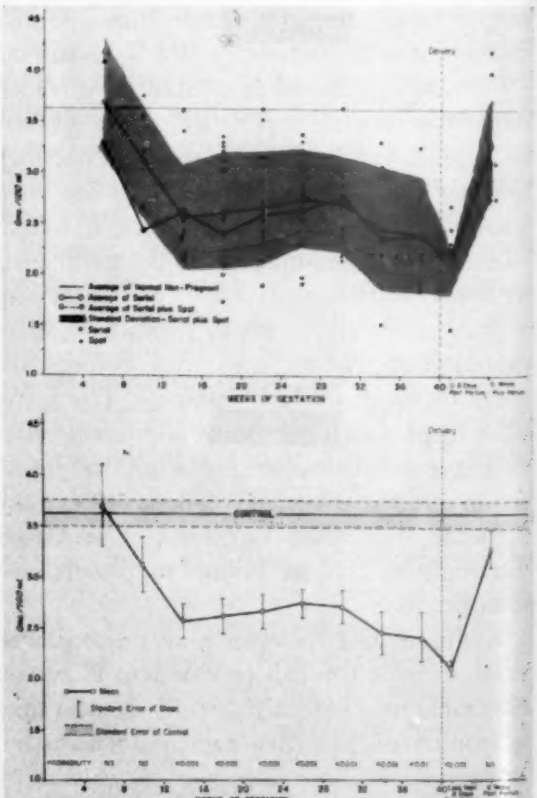


**Fig. 2.** ***Serum albumin determined during normal pregnancy.*** All determinations are grouped by weeks of gestation ante partum, and into early and late postpartum groups. The control group is composed of normal, nonpregnant, young adult women.

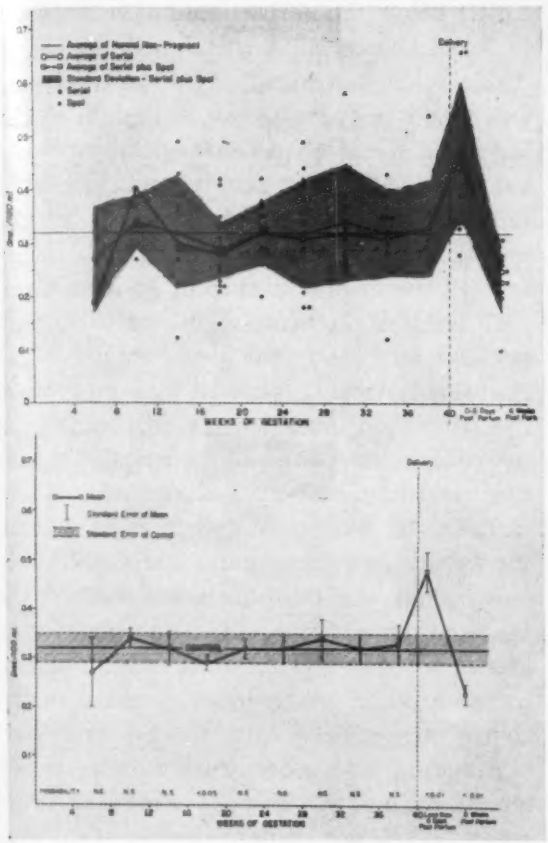

Fig. 3. *Serum alpha$_1$ globulin determined during normal pregnancy.* All determinations are grouped by weeks of gestation ante partum, and into early and late postpartum groups. The control group is composed of normal, nonpregnant, young adult women.

attain more exact analysis of mixtures of substances in serum and because of the ease with which these methods could be applied to clinical problems, with use of even minute amounts of substance, it was felt desirable to undertake a study of the proteins not only in pregnancy but in each of the phases of pregnancy and during the period of involution. It is the purpose of this report to present changes in the total serum proteins and protein fractions as determined by filter paper electrophoresis as well as the changes in fibrinogen in and following normal pregnancy.

## Materials and methods

All studies of total proteins and fractional electrophoresis were determined on serum samples, while fibrinogen determinations were carried out on oxalated whole blood. Total protein was determined by the biuret method[47] with purified human serum albumin used as a standard, the concentration of which was determined by micro-Kjeldahl nitrogen analysis with the factor of 6.25 used to convert it to protein concentration. Fibrinogen was determined by means of Ratnoff and Menzie's method.[48] Electrophoretic separation of serum proteins was carried out by the method of Durrum[49, 50] with use of the hanging strip apparatus supplied in the Spinco Model R paper electrophoresis system. Distribution of the protein was determined after the filter paper strips were stained with bromophenol blue. The amount of dye bound by each of the separated fractions was estimated by direct optical scanning

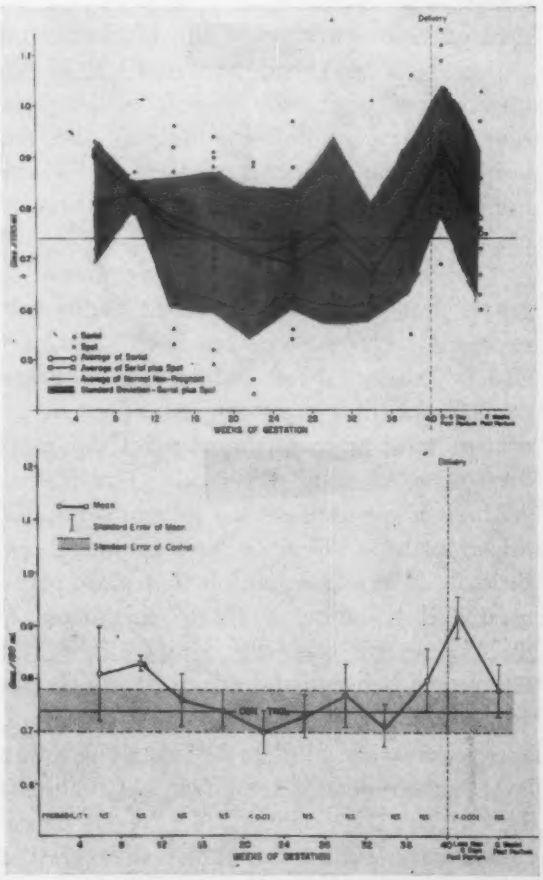

Fig. 4. *Serum alpha$_2$ globulin determined during normal pregnancy.* All determinations are grouped by weeks of gestation ante partum, and into early and late postpartum groups. The control group is composed of normal, nonpregnant, young adult women.

of the strips with the Spinco Analytrol with B-3 cam to record simultaneously the optical density and integral curves.

Control values were obtained of serum total proteins and of protein fractions in 18 healthy nonpregnant women. Plasma fibrinogen control values were obtained in 14 healthy nonpregnant women. All control subjects ranged from 20 to 40 years of age. Determinations of serum total proteins and the various protein fractions were made in 28 normal pregnant women, 11 of whom were followed serially with 6 to 11 determinations. Of the remainder, 2 patients had 3, 1 patient had 2, and 14 patients had a single determination of total and fractionated proteins. Thus, a total of 101 measurements were made. All values were grouped and tabulated according to duration of pregnancy in weeks. The duration of pregnancy was estimated from the date of the last menstrual period but additional confirmation of the duration of pregnancy took into consideration the date of delivery and the size and weight of the infant. A total of 165 determinations of plasma fibrinogen were studied in 150 normal pregnant women.

The serum total proteins were expressed in grams per 100 ml. of serum. In addition to expressing the protein fractions (albumin, alpha-1, alpha-2, beta, and gamma) as grams per 100 ml. of serum, determinations as per cent of total protein (grams per 100 Gm. of total protein) were also made. The fibrinogen values are represented as grams per 100 ml. of plasma. All data were evaluated statistically. After determining that there was a normal distribution of results, by means of the chi square test, the significance of the differences between the means of the control group and of the normal pregnant group was appraised by the Student "t" test. The lowest level of significance used was a P value of 0.05 or less. The symbol "N.S." (not significant) was used in the tables to express P values greater than 0.05.

## Results

**Serum total proteins.** In normal pregnancy the serum total proteins decrease significantly below the normal nonpregnancy level of 7.182 Gm. per 100 ml. of serum (Fig. 1, Table I). A statistically significant decrease was noted first during the thirteenth to the sixteenth week of pregnancy falling to a value of 5.624 Gm. per 100 ml. Once the reduction occurred, it was sustained with the lowest levels (5.592 Gm. per 100 ml.) being found between the seventeeth and the twentieth week. Values rose slightly to 6.013 Gm. per 100 ml. during the twenty-ninth to the thirty-second week, followed by a progressive rise from then until delivery, but these relative differences were not statistically significant. During the first 6 postpartum days and at the sixth and seventh week after delivery the values were consistently and significantly lower than the concentrations observed in the nonpregnant group. The values observed at the sixth to seventh week also were significantly higher than those obtained in the course of pregnancy after the first trimester.

**Albumin.** The concentration of serum albumin during the first 12 weeks of pregnancy does not differ significantly from normal nonpregnant control values (Fig. 2, Table II). During this same period, some investigators describe a significantly reduced serum concentration of albumin[51-56, 60, 62-64] while others show it to be higher[57, 58] than the nonpregnancy level. In our group, a statistically significant decrease in serum albumin concentration begins during the thirteenth to the sixteenth week and remains so throughout the remainder of pregnancy and during the first 6 days of the puerperium. Whether the values are expressed as grams per 100 ml. or grams per 100 Gm. of total protein (per cent), the pattern is similar. The hypoalbuminemia seen in pregnancy is similar to that seen in protein insufficiency due to severe malnutrition and would suggest a rational basis for high protein intake during pregnancy. Normal nonpregnancy levels were attained by the sixth or seventh week postpartum.

**Alpha-1 globulin.** Very little variation from normal nonpregnancy control levels occurs in the concentration of serum alpha-1 globulin during normal pregnancy (Fig. 3,

Table I. Total proteins (grams per 100 ml. of serum) and fibrinogen (grams per 100 ml. of plasma) in normal pregnancy

| Period | Total proteins | | | | Fibrinogen | | | |
|---|---|---|---|---|---|---|---|---|
| | No. | Mean | S.D. | Probability | No. | Mean | S.D. | Probability |
| Control | 18 | 7.182 | 0.596 | – | 14 | 0.256 | 0.058 | – |
| 4- 8 weeks | 2 | 7.270 | 0.184 | N.S. | 5 | 0.260 | 0.022 | N.S. |
| 9-12 weeks | 5 | 6.612 | 0.566 | N.S. | 5 | 0.318 | 0.036 | <0.02 |
| 13-16 weeks | 10 | 5.624 | 0.744 | <0.001 | 8 | 0.309 | 0.044 | <0.02 |
| 17-20 weeks | 14 | 5.592 | 0.867 | <0.001 | 7 | 0.320 | 0.011 | <0.001 |
| 21-24 weeks | 12 | 5.743 | 0.821 | <0.001 | 8 | 0.321 | 0.053 | <0.02 |
| 25-28 weeks | 12 | 5.959 | 0.635 | <0.001 | 14 | 0.366 | 0.084 | <0.001 |
| 29-32 weeks | 9 | 6.013 | 0.678 | <0.001 | 8 | 0.349 | 0.076 | <0.02 |
| 33-36 weeks | 10 | 5.736 | 0.891 | <0.001 | 22 | 0.348 | 0.062 | <0.001 |
| 37-40 weeks | 7 | 5.896 | 0.620 | <0.01 | 26 | 0.379 | 0.073 | <0.001 |
| 1- 6 days postpartum | 11 | 6.015 | 0.579 | <0.001 | 35 | 0.390 | 0.068 | <0.001 |
| 6- 7 weeks postpartum | 9 | 6.613 | 0.305 | <0.001 | 27 | 0.266 | 0.014 | N.S. |

Table II. Serum albumin in normal pregnancy

| Period | No. | Grams per 100 ml. of serum | | | Grams per 100 Gm. of protein | | |
|---|---|---|---|---|---|---|---|
| | | Mean | S.D. | Probability | Mean | S.D. | Probability |
| Control | 18 | 3.621 | 0.585 | -- | 50.2 | 5.7 | -- |
| 4- 8 weeks | 2 | 3.690 | 0.566 | N.S. | 50.7 | 6.4 | N.S. |
| 9-12 weeks | 5 | 3.122 | 0.564 | N.S. | 46.8 | 5.3 | N.S. |
| 13-16 weeks | 10 | 2.570 | 0.513 | <0.001 | 45.6 | 5.5 | 0.01<P<0.05 |
| 17-20 weeks | 14 | 2.621 | 0.578 | <0.001 | 45.7 | 5.4 | 0.01<P<0.05 |
| 21-24 weeks | 12 | 2.669 | 0.551 | <0.001 | 45.5 | 5.2 | 0.01<P<0.05 |
| 25-28 weeks | 12 | 2.749 | 0.505 | <0.001 | 45.9 | 4.1 | 0.01<P<0.05 |
| 29-32 weeks | 9 | 2.718 | 0.477 | <0.001 | 45.2 | 5.7 | 0.01<P<0.05 |
| 33-36 weeks | 10 | 2.449 | 0.598 | <0.001 | 42.3 | 5.3 | <0.01 |
| 37-40 weeks | 7 | 2.396 | 0.580 | <0.01 | 40.4 | 5.5 | <0.01 |
| 1- 6 days postpartum | 11 | 2.210 | 0.192 | <0.001 | 36.7 | 2.7 | <0.01 |
| 6- 7 weeks postpartum | 9 | 3.270 | 0.501 | N.S. | 49.4 | 6.0 | N.S. |

Table III. Serum alpha-1 globulin in normal pregnancy

| Period | No. | Grams per 100 ml. of serum | | | Grams per 100 Gm. of protein | | |
|---|---|---|---|---|---|---|---|
| | | Mean | S.D. | Probability | Mean | S.D. | Probability |
| Control | 18 | 0.320 | 0.066 | -- | 4.5 | 0.19 | -- |
| 4- 8 weeks | 2 | 0.265 | 0.105 | N.S. | 3.7 | 1.06 | N.S. |
| 9-12 weeks | 5 | 0.340 | 0.050 | N.S. | 5.2 | 0.49 | N.S. |
| 13-16 weeks | 10 | 0.323 | 0.107 | N.S. | 5.7 | 0.52 | 0.01<P<0.05 |
| 17-20 weeks | 14 | 0.289 | 0.050 | <0.05 | 5.3 | 0.42 | <0.01 |
| 21-24 weeks | 12 | 0.315 | 0.060 | N.S. | 5.6 | 0.38 | <0.01 |
| 25-28 weeks | 12 | 0.324 | 0.105 | N.S. | 5.6 | 0.35 | <0.01 |
| 29-32 weeks | 9 | 0.338 | 0.103 | N.S. | 5.6 | 0.40 | 0.01<P<0.05 |
| 33-36 weeks | 10 | 0.320 | 0.087 | N.S. | 5.6 | 0.44 | 0.01<P<0.05 |
| 37-40 weeks | 7 | 0.331 | 0.098 | N.S. | 5.6 | 0.57 | 0.01<P<0.05 |
| 1- 6 days post partum | 11 | 0.476 | 0.131 | N.S. | 7.9 | 0.60 | <0.01 |
| 6- 7 weeks post partum | 9 | 0.234 | 0.060 | <0.01 | 3.6 | 0.63 | N.S. |

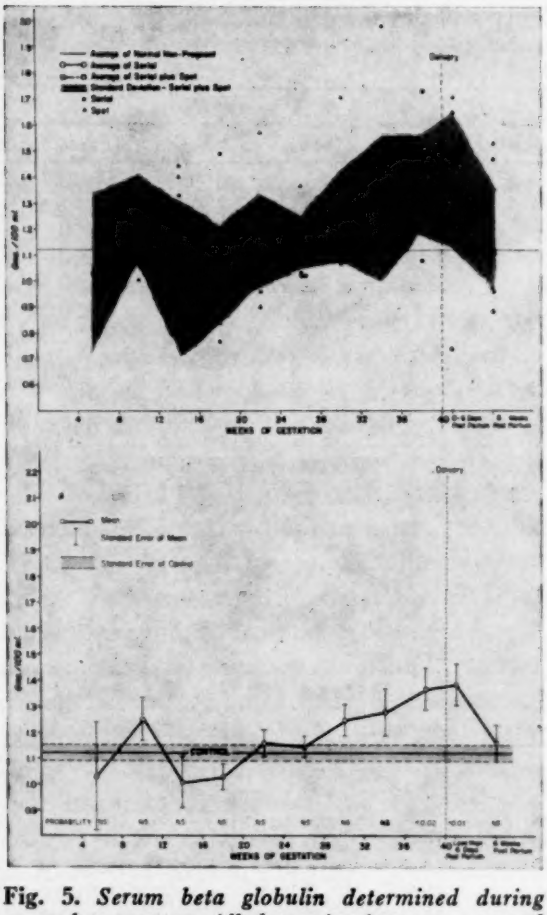

**Fig. 5.** ***Serum beta globulin determined during normal pregnancy.*** **All determinations are grouped by weeks of gestation ante partum, and into early and late postpartum groups. The control group is composed of normal, nonpregnant, young adult women.**

Table III). The normal nonpregnancy level is 0.320 Gm. per 100 ml. of serum. Only once (seventeenth to twentieth week) was there any obvious alteration when the serum concentration fell to 0.289 Gm. per 100 ml. ($P < 0.05$). With this exception, the average values for the remainder of pregnancy remained within the normal nonpregnancy range. A significant postpartum rise during the first 6 days after delivery (to 0.45 Gm. per 100 ml.) occurred. The low average value at 6 weeks post partum was 0.234 Gm. per 100 ml. The value of this globulin expressed as per cent of total protein was increased during the first 6 days of the puerperium and returned to the nonpregnancy levels 6 to 7 weeks after delivery.

**Alpha-2 globulin.** During normal pregnancy essentially no change in serum alpha-2 globulin concentration occurs from the nonpregnancy control values except for a rise at 9 to 12 weeks, when it reaches a concentration of 0.833 Gm. per 100 ml. of serum (Fig. 4, Table IV). Expressed as per cent of total protein, values are higher in pregnant women than in nonpregnant controls. In the first 6 days of the puerperium, the serum concentration of alpha-2 also rose, reaching 0.924 Gm. per 100 ml., but returned to normal nonpregnancy levels at the sixth to seventh week after delivery.

**Beta globulin.** In normal pregnancy our data indicate no change in the serum concentration of beta globulin from normal nonpregnancy values until the thirty-seventh to the fortieth week of pregnancy (Fig. 5, Table V). At that time an abrupt and statistically significant rise to 1.369 Gm. per 100 ml. occurs. This elevation is sustained through delivery and the first week post partum but returns to normal nonpregnancy values by the sixth week after delivery. When expressed as grams per 100 Gm. of protein, a significant rise begins and is sustained after the eighth week of pregnancy.

**Gamma globulin.** In our group the average value for gamma globulin concentration in the normal nonpregnancy control group was 1.378 Gm. per 100 ml. of serum (Fig. 6, Table VI). It will be noted that gamma globulin concentration was not statistically significantly altered during the first 8 weeks of pregnancy. However, at the ninth to twelfth week of pregnancy, gamma globulin decreased to 1.064 Gm. per 100 ml. These reduced levels were sustained throughout the remainder of pregnancy and during the early puerperium but returned toward normal nonpregnancy levels by the sixth week post partum, although still somewhat lower than the normal nonpregnant control group. Expressed as grams per 100 Gm. of protein, gamma globulin maintained normal nonpregnancy values throughout pregnancy except at the twenty-first to the twenty-fourth week and twenty-ninth to thirty-second when values were significantly reduced.

**Fibrinogen.** Increases in plasma fibrinogen occur early in normal pregnancy reaching a

mean value of 0.318 Gm. per 100 ml. of plasma at the ninth to the twelfth week (Fig. 7, Table I). The control level was 0.250 Gm. per 100 ml. The elevation in plasma fibrinogen is sustained throughout all of pregnancy and during the early puerperium, returning to normal nonpregnancy average levels by the sixth week after delivery.

### Comment

It is already obvious in reviewing the subject of protein metabolism during pregnancy that many inconsistencies in trends, actual values, and interpretation of data exist. In order to evaluate these incongruities, it is necessary to consider differences in methodology and the number of cases studied and particularly to determine whether statistical analytical methods have been applied to the results obtained. Many conclusions are based on averages alone without statistical and probability treatment of data. Too few determinations[57, 58, 61, 62, 71-75] and the failure to subject data to statistical analysis[56-59, 63, 71-76] constitute the greatest tribulations in assessing most reports. Other inconsistencies apply to comparison of the medium studied. Some authors studied plasma proteins[51-54, 73, 76] while others studied serum proteins.[55-63, 69, 70, 71, 74] Differences between serum and plasma proteins should be confined to fibrinogen and conceivably to other proteins involved in the clotting mechanism. The findings of Dole and Braun[77] suggest higher concentration of beta and gamma globulin and lower concentration of alpha-1 globulin in serum than in plasma while others[18, 51] found no difference in the concentration of the protein fractions in both media.

Earlier studies of plasma proteins were carried out by means of free boundary electrophoresis[51-54, 62, 72-74, 76] while the more recent measurements have been done with paper electrophoresis.[55-60, 62, 69, 70, 74] Comparisons by us and others[78] of free boundary and paper electrophoresis on the same individuals reveal quite close agreement. Constant modifications in paper are being made to demonstrate more clearly the smallest fractions and to reduce adsorption or "trailing"[78] noted in some fractions.

The finding of lowered serum total protein concentration in pregnancy has been confirmed by most investigators,[51-64] but it is not until one considers changes in total protein throughout the course of pregnancy that disagreement arises. The difference in serum total protein concentration during the first 12 weeks of pregnancy and the values of the normal nonpregnant control is not statistically significant and agrees with other reports.[59, 60, 64]

It is seen that our data indicate a rather precipitous fall in concentration between the twelfth and the sixteenth week of pregnancy which is sustained throughout the remainder

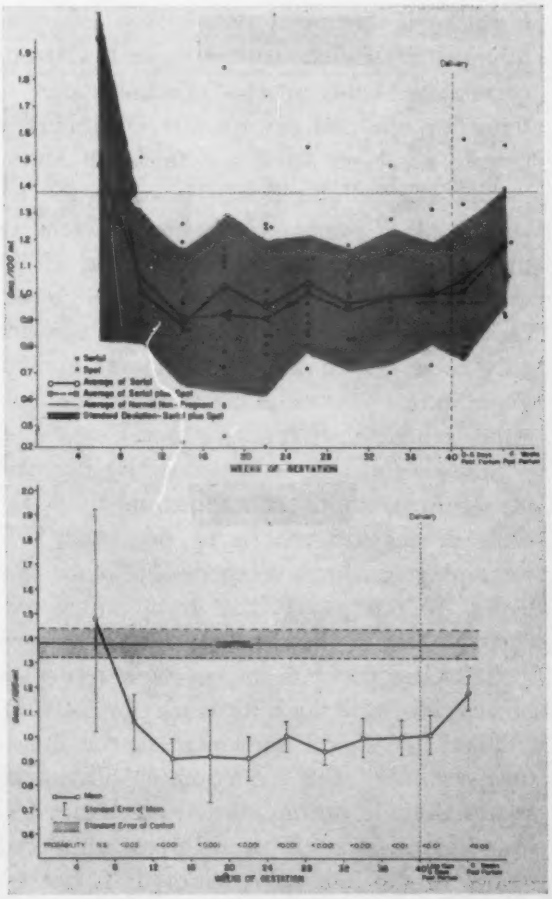

**Fig. 6.** *Serum gamma globulin determined during normal pregnancy.* All determinations are grouped by weeks of gestation ante partum, and into early and late postpartum groups. The control group is composed of normal, nonpregnant, young adult women.

of pregnancy. This differs from some reports[51-54, 61, 63] which describe a continuous fall in serum total protein concentration throughout pregnancy. On the other hand, some reports[56, 62, 64] show a continuous increase during the last trimester of pregnancy. In our series the reduced values of late pregnancy persisted through the first 6 days of the puerperium, but by the sixth or seventh week post partum these values were well on their way toward those of the control group but were still statistically significantly lower than those of the control group.

Paaby[62] and Pfau[55] reported a fall in total protein concentration during the first 12 hours after delivery with a return to preconceptional levels by the sixth week post partum. Bernstein[65] and Paaby[62] feel that the fall in serum total protein concentration during normal pregnancy may be due simply to dilution, inasmuch as they found a strong correlation between the concentrations of serum protein and serum water. The dilution feature has been attributed to an expansion of the extracellular fluid. However, the extent of plasma expansion is not indicated by the reduction in the serum proteins. If the total protein content of the blood is decreased in normal pregnancy, this can be due only to a fall in the albumin and gamma globulin fractions since the other components either remain unchanged or increase.

Some authors report a progressive decrease in serum albumin concentration until the sixth or seventh month of pregnancy,[60, 62] remaining unchanged thereafter until delivery. In our group, the lowest values occurred during the first 6 puerperal days (2.210 Gm. per 100 ml.) with a return to normal levels by the sixth week post partum.

There is general agreement in the literature regarding the reduction of plasma or serum albumin during normal pregnancy followed by an increase toward preconceptional values in the late puerperium. It is not established, however, whether the fall in albumin concentration actually represents decreased, increased, or unchanged total albumin content of the blood. If dilution constitutes the only variable factor and the amount of albumin remains unchanged, then for a percentage decrease in albumin concentration there would be a proportional increase in plasma volume.

Depending upon whether the increase in plasma volume in normal pregnancy is 49 per cent,[66] 22 per cent,[67] or 50 to 65 per cent,[68] the 33 per cent decrease in albumin concentration would represent, respectively, unchanged, decreased, or increased total circulating albumin. The observation of decreased albumin, when expressed in grams per 100 Gm. of protein, would favor a decrease in total circulating albumin. A definite answer may be obtained if concomitant measurements of albumin concentration and plasma volume are made throughout pregnancy. Mack, in his fine reports,[51-54] indicated several possible explanations for the decrease of albuminemia during normal pregnancy.

Reports of concentration of alpha-2 globulin also show variation in concentration. Some report an increase[51-54, 59, 60, 68] during normal pregnancy. Some state that the increase is progressive throughout pregnancy,[51-54, 60, 61] while others have noted very slight changes or even a slight reduction in concentration in the first trimester followed by a progressive rise.[57, 58] In our study a slight rise was noted in the concentration of serum alpha-2 globulin (0.833 Gm. per 100 ml. of serum); this was, however, not statistically significant. When expressed as per cent of total protein, values are higher in the pregnant woman than in the nonpregnant control. During the first 6 days of the puerperium, the serum concentration of alpha-2 globulin reached levels higher than any during pregnancy and significantly higher than the nonpregnancy values, but by the sixth week post partum these values had returned to those of the normal nonpregnant control group. The high value immediately post partum was 0.924 Gm. per 100 ml.

In normal pregnancy the concentrations of alpha-1 and alpha-2 globulins were not significantly different from normal nonpregnancy control values. In the presence of the known increase in plasma volume in preg-

Table IV. Serum alpha-2 globulin in normal pregnancy

| Period | No. | Grams per 100 ml. of serum | | | Grams per 100 Gm. of protein | | |
|---|---|---|---|---|---|---|---|
| | | Mean | S.D. | Probability | Mean | S.D. | Probability |
| Control | 18 | 0.740 | 0.164 | -- | 10.4 | 0.18 | -- |
| 4- 8 weeks | 2 | 0.810 | 0.126 | N.S. | 11.3 | 1.56 | N.S. |
| 9-12 weeks | 5 | 0.833 | 0.029 | <0.01 | 12.6 | 0.36 | <0.01 |
| 13-16 weeks | 10 | 0.755 | 0.156 | N.S. | 13.5 | 0.85 | <0.01 |
| 17-20 weeks | 14 | 0.736 | 0.142 | N.S. | 13.2 | 0.53 | <0.01 |
| 21-24 weeks | 12 | 0.703 | 0.149 | N.S. | 12.3 | 0.75 | <0.01 |
| 25-28 weeks | 12 | 0.727 | 0.121 | N.S. | 12.2 | 0.52 | <0.01 |
| 29-32 weeks | 9 | 0.770 | 0.187 | N.S. | 12.8 | 0.73 | <0.01 |
| 33-36 weeks | 10 | 0.705 | 0.124 | N.S. | 12.5 | 0.57 | <0.01 |
| 37-40 weeks | 7 | 0.800 | 0.157 | N.S. | 13.6 | 0.76 | <0.01 |
| 1- 6 days post partum | 11 | 0.924 | 0.103 | <0.001 | 15.4 | 0.48 | <0.01 |
| 6- 7 weeks post partum | 9 | 0.777 | 0.149 | N.S. | 11.8 | 0.78 | 0.01<P<0.05 |

Table V. Serum beta globulin in normal pregnancy

| Period | No. | Grams per 100 ml. of serum | | | Grams per 100 Gm. of protein | | |
|---|---|---|---|---|---|---|---|
| | | Mean | S.D. | Probability | Mean | S.D. | Probability |
| Control | 18 | 1.124 | 0.145 | -- | 15.8 | 0.59 | -- |
| 4- 8 weeks | 2 | 1.030 | 0.311 | N.S. | 14.2 | 2.84 | N.S. |
| 9-12 weeks | 5 | 1.254 | 0.168 | N.S. | 19.1 | 1.52 | 0.01<P<0.05 |
| 13-16 weeks | 10 | 1.013 | 0.327 | N.S. | 18.9 | 1.08 | 0.01<P<0.05 |
| 17-20 weeks | 14 | 1.031 | 0.188 | N.S. | 18.7 | 1.02 | 0.01<P<0.05 |
| 21-24 weeks | 12 | 1.160 | 0.176 | N.S. | 20.4 | 0.84 | <0.01 |
| 25-28 weeks | 12 | 1.148 | 0.101 | N.S. | 19.5 | 0.58 | <0.01 |
| 29-32 weeks | 9 | 1.247 | 0.190 | N.S. | 20.9 | 0.90 | <0.01 |
| 33-36 weeks | 10 | 1.277 | 0.276 | N.S. | 22.3 | 0.95 | <0.01 |
| 37-40 weeks | 7 | 1.369 | 0.199 | <0.02 | 23.4 | 1.29 | <0.01 |
| 1- 6 days post partum | 11 | 1.387 | 0.258 | <0.02 | 22.9 | 0.88 | <0.01 |
| 6- 7 weeks post partum | 9 | 1.157 | 0.222 | N.S. | 17.5 | 1.17 | N.S. |

Table VI. Serum gamma globulin in normal pregnancy

| Period | No. | Grams per 100 ml. of serum | | | Grams per 100 Gm. of protein | | |
|---|---|---|---|---|---|---|---|
| | | Mean | S.D. | Probability | Mean | S.D. | Probability |
| Control | 18 | 1.378 | 0.248 | -- | 19.3 | 0.84 | -- |
| 4- 8 weeks | 2 | 1.480 | 0.665 | N.S. | 20.5 | 6.80 | N.S. |
| 9-12 weeks | 5 | 1.064 | 0.252 | <0.05 | 16.2 | 1.48 | N.S. |
| 13-16 weeks | 10 | 0.912 | 0.263 | <0.001 | 16.3 | 1.52 | N.S. |
| 17-20 weeks | 14 | 0.914 | 0.381 | <0.001 | 16.1 | 1.44 | N.S. |
| 21-24 weeks | 12 | 0.903 | 0.296 | <0.001 | 15.6 | 1.33 | 0.01<P<0.05 |
| 25-28 weeks | 12 | 1.000 | 0.223 | <0.001 | 16.9 | 1.16 | N.S. |
| 29-32 weeks | 9 | 9.941 | 0.162 | <0.001 | 15.7 | 0.83 | 0.01<P<0.05 |
| 33-36 weeks | 10 | 0.985 | 0.249 | <0.001 | 17.4 | 1.42 | N.S. |
| 37-40 weeks | 7 | 1.001 | 0.194 | <0.01 | 17.1 | 1.36 | N.S. |
| 1- 6 days post partum | 11 | 1.016 | 0.262 | <0.01 | 17.2 | 1.78 | N.S. |
| 6- 7 weeks post partum | 9 | 1.176 | 0.222 | <0.05 | 17.8 | 1.17 | N.S. |

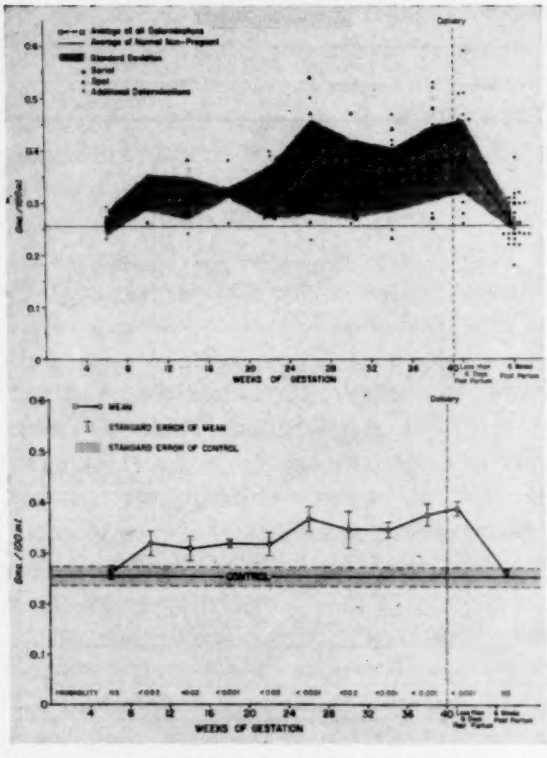


**Fig. 7.** ***Plasma fibrinogen determined during normal pregnancy.*** All determinations are grouped by weeks of gestation ante partum and into early and late postpartum groups. The control group is composed of normal, nonpregnant women.

nancy the unchanged concentration of these globulins represents an increase in their total circulating amount. The increase in these two fractions when expressed as percentage of total protein may reflect this total increase as well as the decrease in albumin and gamma globulin.

The fact that the alpha globulins do not change with advancing pregnancy may be regarded as a relative increase inasmuch as the major protein constituents drop so pronouncedly. This plus the increase in beta globulins in pregnancy is probably explained by the functions that they perform. There are several alpha proteins with known functions that are increased in pregnancy such as ceruloplasmin,[80] transcortin,[81] prothrombin,[82-85] hypertensinogen,[53] alkaline phosphatase,[53] thyroxin-binding protein,[86] and alpha lipoproteins.[87] The haptoglobins are, according to present methodology, quantitatively unchanged during pregnancy[88] but, since almost all changes in the alpha-2 fraction are associated with haptoglobin changes,[88] it is difficult to understand the increased alpha-2 levels in pregnancy reported by others.[51-5; 59, 60, 68]

By the use of vertical starch-gel electrophoresis, new proteins have been found in the blood of pregnant women. These are probably alpha-2 globulins and consist of the pregnancy zone[109] and cystine aminopeptidase 1 and 2 that represent oxytocinase.[110] The appearance of these proteins in the blood of pregnant women may account for an increase in alpha-2 globulins, but some controversy exists as to whether there is an increase in total alpha-2 globulins or an increase in concentration of this fraction.

In general, beta lipoproteins[87] and transferrins[89-91] are increased in pregnancy, accounting for the increase in beta globulin fraction that has been reported. As with all other fractions, the serum or plasma beta globulin concentration has been reported to increase progressively throughout pregnancy by some[56, 60-63] and to decrease[51-54, 57, 58] or remain unchanged[59, 68] during the first trimester and increase during the subsequent course of gestation by others.[51-54, 57-59, 68] As noted in Figs. 9 and 10, there was no change in our patients until the twenty-eighth week of pregnancy when the concentration of beta globulin began to rise gradually until statistically significantly higher concentrations were reached just prior to term. The difference in the beta globulin concentration found in our study and in those where free boundary electrophoresis was used may well be due to the increased lipemia of pregnancy.[79] Falsely low values of beta globulin are found in lipemic sera when paper electrophoresis is employed.[78]

Most authors describe a decrease in gamma globulin concentration in the serum of normal pregnant women[51-54, 56-58, 60, 63] while others indicate that there is no change from the nonpregnancy average values.[55, 59, 60, 61, 68] Only 2 authors report a possible increase during normal pregnancy.[55, 59] Among those reporting a decrease the only disagreement is whether this is or is not present during the first trimester of pregnancy. Fetal gamma

globulin is derived entirely from the mother. Even though the other protein fractions may be synthesized by the fetal liver, neither the fetus nor the placenta has been found capable of synthesizing gamma globulin.[9, 10, 15, 92]

The depression in gamma globulin found in the mother is partly due to a gamma globulin "vacuum" presented by the fetus. This, together with the total metabolic stress of pregnancy, probably results in an inability of the overstressed mechanisms for gamma globulin and total protein synthesis to maintain normal nonpregnancy levels.

Our findings in fibrinogen concentration during normal pregnancy are in agreement with Mack's reports.[51-54] Even though the increase is progressive throughout pregnancy and statistically significantly different from the average value in the nonpregnant control, comparison among the increased values, once they occurred, was not statistically significant. In addition to fibrinogen, it has been repeatedly demonstrated that the activities of prothrombin[82-85] and serum prothrombin accelerator[83, 93] are increased during pregnancy. Since these constituents of the blood are protein in nature,[94] we must consider the changes that take place in the coagulation mechanisms in pregnancy. Studies of the blood platelets in pregnancy, labor, and the puerperium have been inconclusive. Some reports indicate a rise,[95] others a fall[96] and still others report that the platelets are unchanged.[97] Other factors, thrombin time for instance, show no change.[84] In the early puerperium, however, there is rapid fibrinolysis[84] with concomitant shortened clot lysis time.[98] Despite all these reported changes in clotting factors, no significant change occurs in the clotting time in normal pregnancy.[99] Thus, it would seem that the changes in clotting factors which do take place in pregnancy merely represent compensatory alterations to maintain a normal clotting time, bringing about changes in all anticoagulant factors during normal pregnancy. Likewise, the changes in these protein factors probably contribute to the maintenance of a constant level or elevation of the alpha and beta globulins.

Reflecting on the whole spectrum of protein changes which occur in pregnancy we were led to examine clinical medicine to see whether analogous patterns of protein fractionation appeared in other problems. Human growth bears some resemblance to childbearing in regard to serum protein changes. During the first 3 months of the newborn infant's growth there are depression of albumin and gamma-globulin and elevation of the alpha-2 and beta globulin much as one sees during the course of normal pregnancy.[100] The change in alpha-2 globulin is small during this period of infancy just as during pregnancy. The fibrinogen decreases during the first year of life.[101] It rises progressively during childhood, but this increase is not as marked as that seen in pregnancy.[101] Nephrosis[18, 102] is associated with some serum protein alterations similar to those encountered in pregnancy with depressed albumin and gamma globulin levels and elevated fibrinogen; however, the beta globulin is unchanged and the alpha-1 is slightly depressed while the alpha-2 is markedly increased. The Cushing syndrome[103] may produce protein changes much like those of pregnancy with decreased albumin and gamma globulin concentrations, slightly increased alpha-2, but little change in the other fractions. Cortisone administration[104] produces similar changes and the elevation of glucocorticoids in pregnancy is well known.[105-107] No elevation of glucocorticoids is noted, however, during childhood or in nephrosis.[108]

The meaning of the similarities found in the serum proteins in normal pregnancy, infancy, nephrosis, and Cushing's syndrome is not clear, but, as with other studies, the balance between normal and abnormal seems to be extremely tenuous. Just as the clinical picture of toxemia closely resembles that of nephrosis, the electrophoretic distribution of serum proteins in these processes is similar. Certain speculations can be made and some general observations as to the mechanisms involved are apparent, but much detailed work remains necessary before we can comprehend adequately the meaning of known and some unknown blood proteins observed

in pregnancy or indeed in any physiologic stress.

## Summary

1. Serum total protein concentration falls in early normal pregnancy and persists at subnormal levels throughout pregnancy.

2. Plasma fibrinogen increases in normal pregnancy.

3. Serial paper electrophoresis of serum proteins during normal pregnancy reveals a decrease of albumin and gamma globulin when compared to normal nonpregnancy values.

4. The serum concentration of alpha-1 and alpha-2 globulins in normal pregnant women does not differ from that of the nonpregnant control group, and thus implies a relative increase during pregnancy.

5. Prior to the thirty-seventh week of normal pregnancy, the serum beta globulin level remains unchanged but a significant rise occurs from the thirty-seventh week to term.

6. The unchanged or increased concentrations of globulin fractions in the presence of increased plasma volume of normal pregnancy really represent increased total circulating content of these proteins and most likely not only reflect increased functions of these partitions but also account for specific proteins peculiar to pregnancy alone.

7. The reduction of the albumin and gamma globulin fractions seems to contribute in large measure to the decrease of serum total protein concentration noted in normal pregnant women.

8. The trend of total protein, alpha-2, beta, gamma globulin, and fibrinogen concentrations in the late puerperium returns toward normal nonpregnancy values.

We wish to acknowledge with sincere appreciation the participation and full cooperation of the following: Dr. Rudolf J. Vracko for translations from the German literature; Dr. Blair M. Bennett for assistance in statistical methods; Miss Gloria E. Bratvold, Research Associate, for assistance in data classification; Miss Donna M. Simkins and Mrs. Pearl Namkung for technical assistance; Mrs. Norma Baker for secretarial assistance in the preparation of the manuscript.

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## Discussion

Dr. E. Stewart Taylor, Denver, Colorado. It has been long recognized that the concentrations of proteins in the serum and plasma change during gestation and the early puerperium. Many of the measured changes are results of dilution of the plasma secondary to the 40 to 60 per cent increase in plasma volume characterizing normal pregnancy. It is thought by many that the physiologic hydremia of pregnancy will account for the drop in concentration in serum albumin and globulin which has been recognized in this study and by others. The reduced concentrations of albumin and globulin in the serum do not necessarily mean that the total plasma content of these proteins is altered.

It is known that rapidly metabolizing malignancies contribute to hypo–gamma globulinemia of the host. In theory this might be analogous to the mother's carrying a fetus and its placenta, and be a second explanation for low gamma globulin levels.

The increased plasma volume, however, does not reduce the concentration of fibrinogen or alpha and beta globulins in a pregnant patient's serum or plasma. It then follows, since these protein fractions are either present in concentrations the same as or greater than those seen in nonpregnant human beings, that the pregnant patient must be producing and circulating an absolute increase of these proteins.

This is a well-performed and well-controlled study. From a technical point of view, I am not competent to judge the method or the calculation of data. Dr. de Alvarez used a P value of 0.05 or less. Many investigators doing this type of work would have used a P value of 0.01 or less in interpreting statistical significances. There is also some doubt that the biuret method for total proteins is accurate to ± 0.1 Gm. per 100 ml. The same may be said of the measurements of the protein fractions. It, therefore, may not be desirable to try to calculate these measurements to the third decimal point; the methods are not that accurate.

The essential differences in results reported in this study compared to those of others has to do with the alpha globulins. Mack found that these fractions were increased during pregnancy, while the present study shows values comparable to those in nonpregnant patients.

Little of proved clinical significance can be said of the alpha or beta globulins in relation to obstetrics. The alpha globulins increase under the stimulation of inflammation and tissue destruction. Prothrombin is one of the alpha globulins. Thyroxin is carried largely by the $alpha_2$ fraction. The alpha and beta proteins are involved with the lipoproteins.

This paper is a confirmation of studies by others except for the differences pointed out

about the alpha globulins. Future studies of protein metabolism are going to have to investigate alterations in total extracellular volume, plasma volume, rates of protein synthesis from tagged amino acid precursors, and the catabolism of protein fractions. Future research will perhaps tell us the meaning of the changes in the blood proteins that occur in pregnancy.

DR. DE ALVAREZ (Closing). We are well aware of some of the problems of methodology in electrophoresis. Electrophoresis is a relatively new means of studying proteins, having been introduced in 1937, but the introduction of paper electrophoresis came in 1950, with many new modifications constantly occurring.

Probably the greatest problem exists in the area of lipoproteins. We would, therefore, be greatly preoccupied with the alpha and beta globulin protein fractions and particularly with the beta fraction inasmuch as this is the protein attached to the lipoprotein with which we in obstetrics and gynecology are most concerned. Since a lipemia of pregnancy occurs as pregnancy advances, there may be abnormal trailing of the beta fractions so that the value obtained may seem considerably disproportionate, and we are well aware that it is necessary to study these findings carefully to determine whether a true separation of each of the fractions is obtained.

There are methods of determining total protein other than the biuret method but they are not very practical. Our standard is determined with the Kjeldahl method. However, this is used only for the determination of the standard and does not apply to each protein determination itself. Fortunately, modern equipment is now being devised so as to make the Kjeldahl determination of nitrogen a less cumbersome and arduous method than that which we all remember using in medical school.

I am interested in what Dr. Taylor has said about hypo–gamma globulinemia associated with malignancy. Presently we have under way an analogous study in patients with gynecologic malignancy and are confirming this observation. We are interested in finding out what happens to protein metabolism in patients with extremely early malignancy. We wonder if the day may not come when we shall be able to predict the development of cancer by determining proteins, their fractions, subfractions, and prosthetic compounds.

Additional work has to be done in protein metabolism as it relates to normal pregnancy and in obstetric and gynecologic disease processes. Not only does this constitute a tremendous area in the study of proteins alone, but there must be simultaneous study in the related areas of interstitial fluid volume, plasma volume, cell volume, protein synthesis, measurement of all other protein fractions such as glycoproteins, etc. When we break down glycoproteins, for example, we get into the area of carbohydrate metabolism and all its fractions, and, similarly, in lipoprotein study, all the fractions of the major lipid fractions. Then subfractionation of the lipid, protein, and carbohydrate fractions all the way down to their elemental constituents, looms as an endless avenue for research.

# Cineradiographic studies of female urinary continence

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CINERADIOGRAPHY offers a unique opportunity for observation of the rapid anatomic and physiologic changes of the female bladder and urethra in states of continence and incontinence. Lund and coauthors in 1957[1] and 1959[2] reported their cineradiographic studies of the urethrovesical relationships during voluntary micturition and with stress incontinence. They demonstrated the significant contribution this technique offers to an improved understanding of bladder and urethral physiology.

Based upon extensive investigation of the physiology of the bladder and urethra in animals and humans, Lapides in 1960[3] reported that urinary continence in the female was dependent upon the resistance created by the length and the tonicity of the smooth muscles and elastic tissues in the wall of the "primary urinary sphincter" (the proximal 3 cm. of the urethra). He further stated that the basic defect in most cases of female urinary incontinence was an abnormal shortening of the urethra when the patient assumed the standing position. He reported that lengthening the urethra by surgical approximation of the anterior wall of the urethra to the overlying symphysis pubis and rectus fascia completely alleviated urinary stress incontinence without regard to coexisting urethrocele, cystocele, or procidentia. Lapides determined the existence of an abnormal shortening of the female urethra by measuring the urethral length with the patient in the standing position with a retention catheter which had circular graduated markings in centimeters for a distance of 5 cm. from the inflated balloon. In 35 female patients with urinary continence, he reported that the urethral length varied from 3 to 5 cm., an average of 3.8 cm. Female patients with urinary stress incontinence, before operation, had urethral lengths of 1.8 to 2.7 cm., an average of 2.3 cm., and, after anterior urethropexy which completely relieved the incontinence, he found the patients to have urethral lengths of 3 to 5 cm., an average of 3.8 cm.

The simplicity of Lapides' concept of the etiology of female urinary stress incontinence and the ease with which the female urethra can be measured with the retention catheter led us to focus our cineradiographic studies of the female bladder and urethra on an investigation of the significance of the length of the urethra in female urinary continence.

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## Methods of investigation

This report is based upon an investigation of the significance of the length of the

urethra as a determining factor in urinary continence in 58 gynecologic patients. None of these patients had evidence of neurological disease, urinary fistula, or inflammatory disease of the bladder or urethra.

Some of the patients had cineradiographic studies only and some had urethral lengths measured by catheter only, but the majority were studied by both methods of investigation so that the findings by each technique could be correlated.

**Cineradiographic studies.** We have performed 60 cineradiographic studies of the bladder and urethra in 42 gynecologic patients. A number of patients had more than one study made. At the time of the examination, 25 patients were continent, 15 patients complained of slight to moderate incontinence, and 16 patients complained of severe stress incontinence.

*Preparation of patients.* The patient was placed in the supine position and catheterized. With the catheter in place, the bladder was slowly filled by gravity to capacity, as determined by the complaint of slight discomfort, with sodium acetrizoate solution (50 c.c. of 70 per cent sodium acetrizoate to each 250 c.c. of sterile water). Usually 400 to 500 c.c. of fluid was instilled. The catheter was then removed.

*Cineradiographic equipment and method.* The cine recordings were made on 16 mm. Lineagraph Shellburst film utilizing an 8 inch electron-type image intensifier over a conventional fluoroscopic table. The equipment included a 16 mm. motor-driven camera focused on the output phosphor of the image tube. Film sequences were made at 7½ frames per second. Exposures were controlled by a center-scan brightness stabilizer which monitored the light from the output phosphor of the image tube. Pulsed x-ray exposure of 1/60 of a second were used. These were synchronized so as not to work during the transit time of the film in the camera. Radiation was generated at 120 kv.

Patients were filmed in the erect posture in the posteroanterior oblique, and lateral projections at rest, straining and coughing, tightening of levator muscles, and during voiding. The film sequences ran from 10 to 60 seconds in length, and the average radiation-skin dose was 2 r per minute for a patient 20 cm. in thickness. Films were processed automatically in medium grain dectol developer. The kinetic motion of the bladder and urethra was studied with a Perceptoscope analyzing projector in slow and fast motion.

It became evident that evaluation of the length of the urethra by cineradiography presented difficulties. The entire length of the urethra could be seen only in the voiding studies and even in some of these the presence of a cystocele obliterated part or all of the urethra, especially in the anterior projection. To aid in identification of the distal end of the urethra, we employed the use of a metal skin clip placed just above the external urinary meatus, after the area had been infiltrated with a small amount of 1 per cent lidocaine solution (Fig. 1). To facilitate outlining the full length of the urethra, we inserted the end of a small metal bead chain (10 cm. long, each bead 0.2 cm. in diameter; 4½ beads = 1 cm. in length) into the bladder, after the bladder had been filled with sodium acetrizoate solution. Because of its extreme pliability, we felt that this chain would cause the minimum of foreign body distortion. While helpful in studying the changes associated with different positions and with straining and coughing,

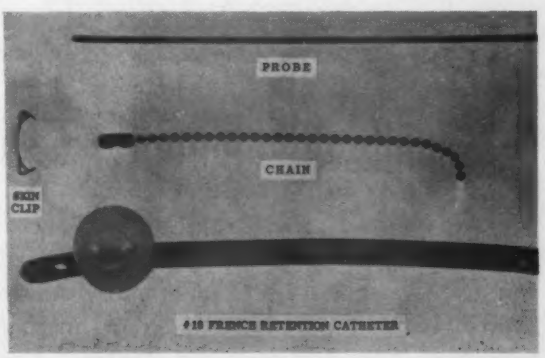



**Fig. 1.** Skin clip, straight probe, and metal bead chain employed to identify location of external urinary meatus and course of urethra in cineradiographic studies. The No. 18 Fr. retention catheter with circular graduated markings in centimeters was employed in measuring the length of the urethra.

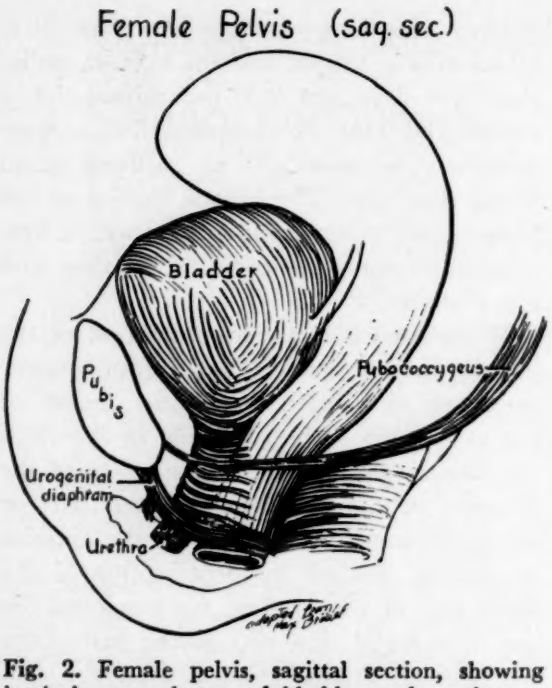



**Fig. 2.** Female pelvis, sagittal section, showing intrinsic musculature of bladder and urethra to be anatomically continuous, functioning as a physiologic unit. The extrinsic fascia and striated musculature hold the distal urethral segment firmly in position while that supporting the proximal urethral segment and bladder base permit considerable mobility.

the chain was invariably expelled as soon as voiding commenced.

We made repeated attempts to obtain lateral projections, first with the patient standing and then with the patient sitting on a plastic bedpan with thighs flexed, but the technical problem of penetrating the bony structures of the pelvis with the currently available cineradiographic equipment proved insurmountable in most cases.

In an endeavor to obtain cineradiographic evidence of the tone of the proximal urethra in the presence of increased intravesical pressure, we inserted a thin-walled rubber balloon into the bladder of several patients and filled the balloon slowly by gravity with sodium acetrizoate solution to the point of bladder capacity. The neck of the balloon was clamped at the external urinary meatus. The changes in the bladder and urethra associated with coughing and straining in the erect position were then observed by cineradiography.

**Studies of the urethral length as measured by catheter.** In 40 of the total number of gynecologic patients studied, we have determined the length of the urethra with the patient in the supine and standing positions in accordance with the technique recommended by Lapides. We have employed a specially prepared No. 18 Fr. retention catheter with circular graduated markings in centimeters for a distance of 5 cm. from the balloon (Fig. 1). The catheter was inserted into the bladder and the bladder emptied. The balloon was inflated with 5 c.c. of sterile water and the catheter then pulled gently until no further descent of the balloon occurred. The urethral length was determined from the centimeter markings on the catheter. Urethral lengths were determined with the patient in the supine position, at rest, on heavy straining, and on tightening of the levator muscles. Urethral lengths were then determined with the patient in the standing position, at rest, and straining and tightening of the levator muscles. Of the 40 urethral measurements determined, 25 patients had no stress incontinence, 9 patients complained of mild to moderate stress incontinence and 6 patients complained of severe stress incontinence.

## Results

**Cineradiographic findings.** From the analysis of our cineradiographic data, it became apparent that from the functional point of view the urethra may be divided into two segments (Fig. 2).

The distal, fixed segment extending from the external urinary meatus to the upper level of the urogenital diaphragm was ap-

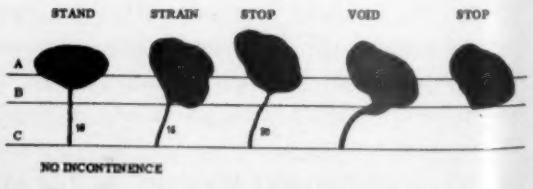
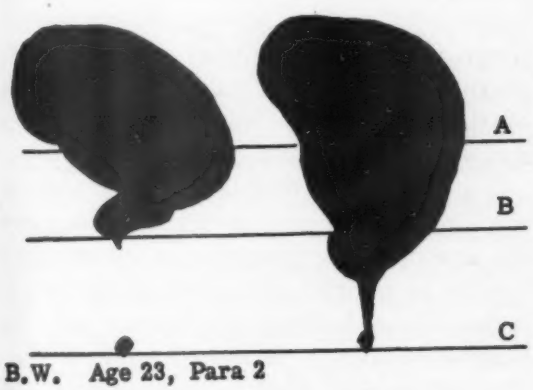



**Fig. 3.** Composite cineradiographs of patients with no stress incontinence. Line *A* is upper border of symphysis pubis; *B* is lower border of symphysis pubis, and *C* is location of external urinary meatus. Numbers indicate number of beads of metal chain.

parent in most cases. This portion was usually 1 to 1.5 cm. in length and appeared to be held firmly in position by its surrounding fascial and striated muscle attachments which extended to the symphysis anteriorly and to the pubic rami laterally. Regardless of the degree of cystocele or the extent of urinary stress incontinence, this fixed, distal segment of the urethra seldom changed in position, length, or lumenal diameter. Its lumen remained closed except to permit the passage of fluid from the bladder during normal micturition or in association with stress incontinence.

The proximal or mobile segment of the female urethra extended from the upper part of the urogenital diaphragm to the bladder neck and, in the continent patients, was found to be 2 to 3.5 cm. in length. It appeared extremely mobile, rapidly changing position along with the bladder base during changes in the patient's position, becoming slightly shortened with straining, lengthening with levator contraction or descending downward in a C- or S-shaped curve in the presence of a large cystocele. During micturition, the proximal urethra appeared to function as an integral part of the musculature of the base of the bladder. In patients with stress incontinence, the simultaneous dilatation, beaking and funneling of the bladder base, and proximal urethra made it apparent that both were intimately involved in the same physiopathologic process.




B.W. Age 23, Para 2


O.S. Age 55, Para 2

Fig. 4. Cineradiographs of bladder and urethra containing thin rubber balloon filled with sodium acetrizoate solution. Figures on left, standing; figures on right, standing and straining. *B.W.*, incompetent bladder neck and proximal urethra. *O.S.*, cystocele with competent bladder neck and proximal urethra.

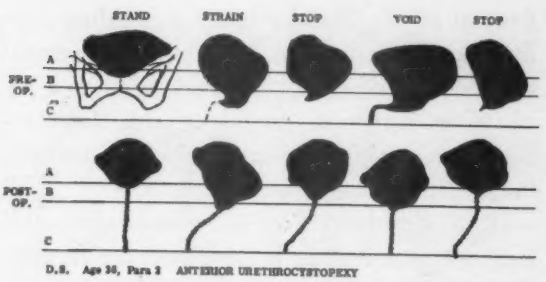



Fig. 5. *D. S.*: Severe stress incontinence. Cineradiographs before and after anterior urethrocystopexy.

*Patients with no stress incontinence.*

PAROUS WOMEN WITH MINIMAL CYSTOCELE (FIG. 3).

Proximal urethra. There appeared to be a slight shortening in length of the urethra as the patient changed from the supine to the standing position, but there was no change in the diameter of the urethral wall. With straining and coughing, there was some angulation observed as the bladder base descended, but the diameter and length of the urethra showed little change. At times with forceful straining a small beak, an extension of that created at the bladder orifice, appeared indicating that fluid had entered the upper part of this urethral segment, but the fluid seldom descended more than half the length of this segment and on the cessation of straining the fluid was promptly return to the bladder. With voiding, there was an active opening of the lumen of the urethra in conjunction with the opening of the urethrovesical orifice and

descent of the bladder base. As voiding continued, the proximal urethral segment seemed to elongate without changing in diameter, becoming confluent with the bladder base making it impossible to identify the urethrovesical junction.

Distal urethra. For the most part, this segment remained in a fixed position in relation to its length and to its anterior posterior and lateral orientation in the supine and standing positions. It showed little change with coughing and straining. The lumen dilated only as it received the stream of urine from the proximal segment. In several cases, however, the length of this segment seemed to increase slightly as the bladder base rose toward the end of micturition.

PAROUS PATIENTS WITH LARGE CYSTOCELE. The existence of a cystocele became much more noticeable in the presence of a full bladder than was the case at the time of the previous gynecologic examination performed with the bladder empty.

Proximal urethra. As the bladder base descended with change from the supine to the standing position and with straining and coughing, the proximal segment often underwent a marked angulation, assuming a C- or S-shaped outline. However, there was no evidence of change of total urethral length. Occasionally, there was a slight beaking with straining, but funneling did not appear.

Evidence of normal tone of the bladder neck and the proximal urethra was further demonstrated by inserting a thin, rubber balloon into the bladder of a patient with a large cystocele. The balloon was filled to bladder capacity slowly by gravity with sodium acetrizoate solution. The neck of the balloon was clamped at the external urinary meatus. With coughing and straining, the tone in the bladder neck and proximal urethra was sufficient to prevent any fluid in the balloon from leaking into the proximal urethra. (Fig. 4, O. S.).

Distal urethra. This segment, remaining in a fixed position regardless of the extent of the cystocele, forced the proximal urethra to undergo considerable angulation as the bladder base descended.

*Patients with severe stress incontinence (Fig. 5).*

PROXIMAL URETHRA. Often the urethra seemed to be of normal length in the supine position. On standing, however, definite shortening and dilatation of the proximal urethra appeared as it participated in the beaking and funneling initiated in the bladder neck. Straining and coughing in the standing position resulted in an accentuation of the funneling to the point of fluid incontinence. In some, the funneling seemed to extend down to the distal urethra. When funneling was present, it was impossible to identify the area of the urethrovesical junction. On contracting the levators after straining, the funnel deformity, filled with fluid, often persisted. With initiation of micturition, the funnel deformity of the bladder neck and proximal urethra simply became exaggerated and a urinary stream flowed freely. On sudden voluntary interruption of micturition, there was a delay of the return of the fluid to the bladder, the fluid remaining in the dilated proximal segment.

To further illustrate the loss of tone of the bladder neck and proximal urethra and the accompanying shortening of the proximal urethra in patients with stress incontinence, a thin rubber balloon was inserted into the bladder of such a patient (Fig. 4, B. W.). The balloon was filled to capacity slowly by gravity with sodium acetrizoate solution and the balloon clamped at the external urinary meatus. A marked and persistent dilatation of the bladder neck and most of the proximal urethra was observed. On straining and coughing, fluid entered the collapsed neck of the balloon in the region of the distal urethra.

DISTAL URETHRA. The distal urethra showed no characteristic change in patients with stress incontinence.

*Patients with mild and moderate stress incontinence.* The primary symptom of these patients was usually related to the degree of cystocele and uterine prolapse present, the complaint of stress incontinence being usually of a secondary importance. The degree of stress incontinence varied intermittently.

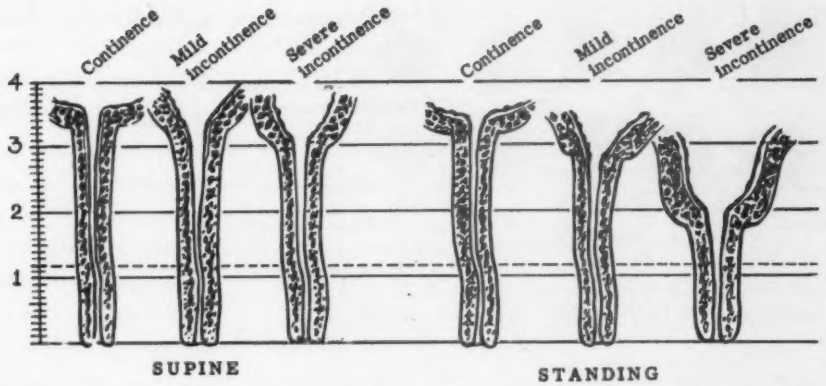



**Fig. 6.** Diagrammatic representation of average urethral lengths of patients as measured by catheter technique in supine and standing positions and correlated with clinical symptoms. Numbers at left are in centimeters. Dotted line represents upper level of urogenital diaphragm.

With illnesses associated with frequent and heavy coughing, stress incontinence became very annoying, while at other times stress incontinence was only occasionally present.

The cineradiographic findings in these patients were intermediate between those with no incontinence and those with severe incontinence. In most, there was a definite though moderate shortening of the proximal urethra and decrease in the tone of the bladder neck and proximal urethra as manifested by frequent beaking and some funneling on straining and coughing in the standing position. Seldom was there any evidence of fluid leakage.

**Urethral length measurements by catheter technique.** The urethral lengths as determined by the catheter technique of Lapides have been correlated with the clinical symptoms and are shown in Table I and Fig. 6.

It will be seen that the greater the degree of stress incontinence, the shorter the urethra as measured with the patient in the standing position. The difference between urethral lengths in the supine and in the standing position shows an increase as the degree of stress incontinence increases.

*No stress incontinence.* Of the 25 patients in this group, none complained of stress incontinence at the time the urethral measurements were determined.

1. Twelve patients had undergone anterior and posterior wall repair and vaginal hysterectomy 4 months to 3 years previously (the anterior wall repair had been performed as described in this presentation). Preoperatively, 2 patients had complained of moderate stress incontinence and 6 patients of marked stress incontinence.

2. Four patients had had Marshall-Marchetti-Krantz procedures for severe stress incontinence a year or more previously.

3. Nine patients had had no previous operation, but currently demonstrated mild to-large cystoceles.

*Moderate stress incontinence.* Each of the 9 patients in this group had varying degrees of cystocele and prolapse. Operation was advised in 3 cases. In the other 6 cases, levator exercises were advised because of the finding that strong levator contractions lengthened the urethras by 0.7 to 1.5 cm. by catheter measurement.

*Severe stress incontinence.* Five of the 6 patients in this group complained primarily of severe stress incontinence. In the sixth patient, the chief complaint was that of a large uterine prolapse.

1. Two patients in the standing position had urethral lengths of 1.5 cm. and 2.5 cm., respectively. With strong levator contractions, each patient could lengthen the urethra by 1.5 cm. Each of these patients was advised on a course of levator exercises.

2. In the other 4 patients, the urethral lengths in the standing position varied between 1.5 and 2 cm. In spite of fair to

**Table I.** Urethral length (catheter measurements) and degree of stress incontinence

| *No. of patients* | *Degree of stress incontinence* | *Supine position* | | *Standing position* | | *Difference (cm.)* |
|---|---|---|---|---|---|---|
| | | *Range (cm.)* | *Average (cm.)* | *Range (cm.)* | *Average (cm.)* | |
| 25 | None | 3.0-5.0 | 3.6 | 2.5-5.0 | 3.4 | 0.2 |
| 9 | Moderate | 3.0-4.0 | 3.3 | 2.0-3.7 | 2.8 | 0.5 |
| 6 | Severe | 2.5-4.0 | 3.1 | 1.2-2.5 | 1.9 | 1.2 |

moderate levator contractions, there was practically no increase in urethral length. Operation was advised for these patients.

The correlation of the degree of stress incontinence in the mild to moderate group with the urethral lengths by catheter measurement and cineradiography was less convincing. There is a more consistent correlation between the degree of incontinence and the length of the urethra by catheter measurement than with that obtained by cineradiography. For example, patients with moderate incontinence clinically had a definitely shortened urethra by catheter measurement, yet on cineradiography showed minimal evidence of shortening of the proximal urethra and no radiographic evidence of stress incontinence. It is our feeling that this discrepancy was more likely because of the minor apprehension and self-consciousness inevitably associated with the preparation for, and the carrying out of, the cineradiographic studies. Patients who had more than one cineradiographic study were able to perform in a much more natural manner in these subsequent studies.

Cineradiographic studies consistently show less evidence of the pathophysiologic disturbance involved than the symptoms of the patient suggest. It is as if the patient underplays the problem in front of the cineradiographic apparatus.

**Surgical procedures for stress incontinence and results.** Based upon the concept that the relief of stress incontinence was dependent upon lengthening and tightening of the proximal urethra by stretching it between the fixed, distal urethral segment and the elevated and anchored bladder base or the elevated and anchored lower anterior bladder wall, surgical procedures were carried out on 21 gynecologic patients whose leading or important secondary complaint was moderate to severe stress incontinence. Preoperative cineradiographic studies had been carried out on each of the 21 patients and 5 had had urethral measurements by catheter. Postoperatively, 15 patients had one or more cineradiographic studies and 13 had had urethral measurements by the catheter technique. The types of surgical procedures employed and the results are shown in Table II.

*Vaginal approach.* The vaginal approach was chosen in the presence of a cystocele and associated rectocele and uterine prolapse. After the lower two thirds of the circumference of the entire proximal urethra had been dissected free and the bladder separated from the vagina and cervix, a No. 18 Fr. retention catheter was inserted into the bladder and the balloon filled with 5 c.c. of sterile water. Pleating of the tissue in the area of the urethrovesical junction was undertaken with mattress sutures, tightening the lumen until traction on the catheter revealed the urethral tube to be at least 4 to 5 cm. in length. Sutures were then taken which elevated and anchored this area of the urethrovesical junction, together with adjacent bladder base, upward and anteriorly. Additional mattress sutures corrected the cystocele and tightened and reinforced the lower part of the proximal urethra. Vaginal hysterectomy and posterior colpoperineorrhaphy was usually performed in addition to the above procedures because of varying degrees of uterine prolapse and rectocele.

This vaginal approach was employed in

14 patients, 10 patients with moderate stress incontinence and 4 patients with severe stress incontinence. There were 13 successes. The average urethral lengths in the standing position of these patients, 6 to 18 months postoperatively, varied between 3 and 4 cm. Postoperative cineradiographic studies of these patients (Figs. 7 and 8) revealed elevation of a more rounded bladder base with less descent on standing, straining, and coughing. The proximal urethra had been lengthened and this increased length persisted in the standing position and also with straining and coughing. Restoration of the caliber of the bladder neck and proximal urethra was evidenced by the absence of the preoperative beaking and funneling on straining or coughing in the standing position, the appearance of a more clearly defined urethrovesical junction, and a decreased dilatation of the vesical orifice and proximal urethra during micturition.

The one failure occurred in a 76-year-old hypertensive patient with a complete uterine prolapse, cystocele, and rectocele. Preoperatively, she complained of moderate stress incontinence and frequently had to replace the uterus in order to be able to void. Operation consisted of a vaginal hysterectomy repair of the cystocele and rectocele, and partial colpocleisis. Careful attention had been paid during the operation to establish a urethra of adequate length and to elevate and anchor the bladder base. However, within 3 months after operation, moderate stress incontinence recurred. Cineradiographic study revealed a recurrence of the same type of funneling of the vesical orifice and proximal urethra and shortening of the proximal urethra as had been seen on cineradiographic study preoperatively. Fourteen months postoperatively, the urethral measurements revealed a standing urethral length of 2 cm. which shortened to 1½ cm. on straining, but increased in length to 3 cm. with strong levator contractions. Clinically, the shortened vaginal vault seemed to be adequately supported. Currently, she is on a regime of levator exercises.

*Suprapubic approach.* The suprapubic ap-

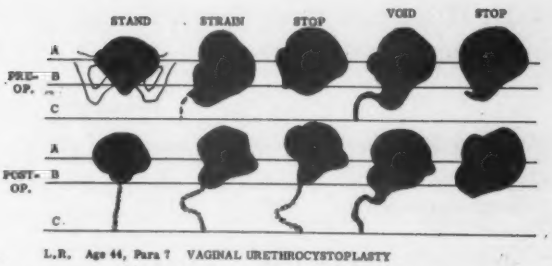



**Fig. 7.** *L. R.* Severe stress incontinence. Cineradiographs before and after vaginal urethrocystoplasty.

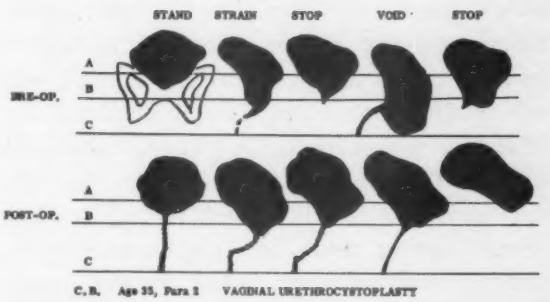



**Fig. 8.** *C. B.* Severe stress incontinence. Cineradiographs before and after vaginal urethrocytoplasty.

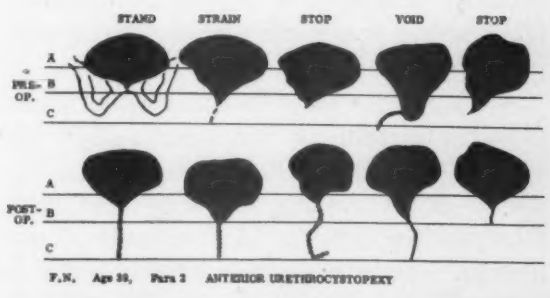



**Fig. 9.** *F. N.* Severe stress incontinence. Cineradiographs before and after anterior urethrocystopexy.

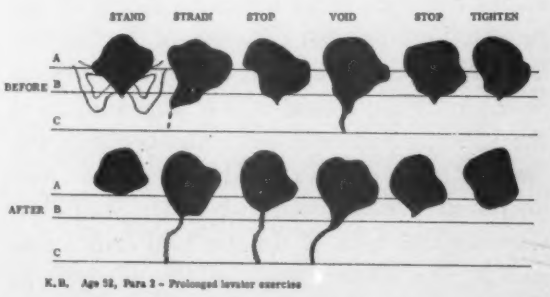



**Fig. 10.** *K. B.* Severe stress incontinence. Cineradiographs before and after prolonged levator exercise.

**Table II.** Surgical procedures for stress incontinence and results

| *Surgical procedure* | *No. of patients* | *Results* | |
|---|---|---|---|
| | | *Success* | *Failure* |
| Vaginal urethrocystoplasty | 14 | 13 | 1 |
| Suprapubic urethrocystopexy | 6 | 6 | 0 |
| Combined | 1 | 1 | 0 |
| Total | 21 | 20 | 1 |

proach was employed in 6 patients, 5 with severe stress incontinence and 1 with moderate stress incontinence. The type of suprapubic anterior urethrocystopexy carried out was essentially the technique described by Marshall and associates.[4, 5] We usually add extra lateral sutures in the area of the urethrovesical junction and several sutures which further pleat and anchor the lower anterior wall of the bladder to the rectus fascia. One patient with severe stress incontinence also demonstrated a moderate cystocele. To ensure adequate bladder base and proximal urethral support for this patient, we performed a vaginal urethrocystoplasty before proceeding with the suprapubic procedure.

All 6 patients have remained either completely continent or continent except for episodes of unusually heavy coughing during the 6 to 18 months following operation. Postoperative catheter measurements of the urethral lengths have been determined in 4 patients and each showed a length of 3 cm. or greater in the standing position.

Postoperative cineradiographic studies were carried out in all 6 patients and revealed evidence of an increased length of the proximal urethra, an elevation of the bladder base and a more well-defined urethrovesical junction (Figs. 5 and 9). It appeared as if the urethra had been stretched between the fixed distal urethra and the fixed and anchored lower anterior bladder wall. On standing, the area of the urethral orifice was still at the lowest point of the bladder in most patients. In the anterior projection, straining in some patients produced the same preoperative broad V-shaped appearance of the bladder base in contrast with the postoperative rounded appearance usually achieved by the vaginal surgical approach. In the oblique projection, straining produced in some beaking of the bladder base and shortening of the proximal urethra, although to a much lesser extent than observed in the preoperative studies.

In the one patient on whom both the vaginal and suprapubic surgical procedures were performed, postoperative cineradiography showed a rounded bladder outline on straining with evidence of good support of the bladder base and no evidence of beaking.

**Effect of levator ani contraction on urethral length.** Strong contractions of the levator musculature elevate the bladder base, lengthening and stretching the urethra. Proof of this was observed repeatedly in our cinegraphic studies by the indentations in the lateral areas of the bladder floor due to contraction of the pubococcygeus muscles which was instantaneously followed by elevation of the bladder base and lengthening of the urethra. This was observed when the patient was instructed to tighten the levator muscles or to interrupt the act of micturition.

Further proof was offered in the urethral length studies by catheter measurement. It was repeatedly observed that contraction of the levator muscles resulted in the catheter being drawn up inside the external urinary meatus for a distance of 0.5 to 1.5 cm.

The permanent benefit with alleviation of urinary stress incontinence which can result from continued levator exercise was demonstrated by one of our patients (Fig. 10):

K. B., 49, para ii, in February, 1958, had had a plastic repair of a cystocele, rectocele, and incomplete uterine prolapse. Preoperatively, she had had no urinary complaints. Two years later, severe stress incontinence developed over a period of several months. Cineradiographic studies revealed evidence of funneling of the bladder neck and proximal urethra with straining with persistence of the funneling when straining was stopped or when she voluntarily interrupted micturition. She began levator exercise and gradually the stress incontinence lessened, then disappeared. A year later, catheter measurements of

the urethra revealed a supine length of 4 cm., a standing length of 3.5 cm., and with levator contraction a urethral length of 4.5 cm. Repeat cineradiography revealed a rounded bladder outline on straining, prompt and exaggerated elevation of the bladder base on levator contraction. However, with coughing and cessation of micturition, a slight beak formation was noted.

**Correlation of urethral lengths as determined by cineradiographic studies and by direct catheter measurements in the standing position.** In the majority of patients in this study, the lengths of the urethras were evaluated by both cineradiography and direct catheter measurements. There was a remarkably good correlation between the findings of the two techniques and the degree of stress incontinence.

Those patients with severe stress incontinence had abnormally short urethras by catheter measurement and marked shortening of the functional length of the urethra on cineradiography because of dilatation of the bladder neck and the upper proximal urethra which became especially apparent on straining and coughing.

Lapides' catheter technique actually measured the functional length of the urethra rather than its anatomic length. The greater the loss of tone of the bladder neck and the proximal urethra, the greater the functional incompetency and the shorter the urethra will measure by the catheter method. Thus, in cases of severe stress incontinence the simple technique of measuring the urethral length in the standing position gave valuable information as to the degree of incompetency of the bladder neck and the upper proximal urethra.

Those patients who had no stress incontinence consistently had urethras of normal length in the standing position as measured by the catheter technique. On cineradiography, they had urethras of good functional length and demonstrated evidence of good tone of the bladder neck and the upper proximal urethra by the fact that, with the patient in the standing position, straining and coughing produced minimal change in urethral length and only ocasionally caused the appearance of a beak at the vesical orifice.

## Comment

This investigation of the significance of the length of the urethra in female urinary continence has been carried out in 58 gynecologic patients by 60 cineradiographic studies and 40 urethral length measurements by catheter technique. To identify the course of the urethra, we have employed a metal bead chain as described by Barnes,[6] Hodgkinson,[7] Kennedy,[8] and Nilsen.[9] We have also employed a skin clip to identify the location of the external urinary meatus.

Our studies reveal that the female urethra is functionally divided into a distal, fixed segment (1 to 1.5 cm. in length) and a proximal and mobile segment (2 to 3.5 cm. in length).

The cineradiographic studies demonstrated that urinary continence was associated with the presence of sufficient intrinsic tone and extrinsic support of the bladder base and proximal urethra to resist sudden high increases in intravesical pressure in the standing position by maintaining a rounded outline of the bladder base and preventing a functional shortening of the proximal urethra. This was also demonstrated by finding urethral lengths as measured directly by catheter technique in the standing position to be consistently greater than 3 cm. in the continent patient.

Our cineradiographic and urethral length studies have shown that the presence of a urethrocele, cystocele, or procidentia, has no apparent relationship to the existence or nonexistence of a functionally shortened urethra and the associated stress incontinence.

Our female patients with severe urinary incontinence, on cineradiographic study, revealed changes consistent with a loss of intrinsic tone and extrinsic support of the bladder base and proximal urethra which have been described by many others,[10-17] namely, a downward descent of the bladder base with the appearance of a funnel deformity of the bladder neck and proximal

urethra when straining or coughing in the standing position. These pathophysiologic changes result in a marked shortening of the functional urethra. Our studies have shown that the more extensive the pathophysiologic change, the greater will be the functional shortening of the urethra and the shorter will be the length of the urethra as measured by direct catheter technique.

Our cineradiographic and urethral length studies have further shown that surgical correction of stress incontinence is dependent upon a lengthening of the functional urethra by elevating and anchoring the bladder base by the vaginal approach or by elevating and anchoring the urethrovesical junction and lower anterior bladder wall to the symphysis and rectus fascia by the suprapubic approach. More permanent lengthening of the urethra is accomplished by pleating and supporting the functional urethra by the vaginal approach or by suturing the paraurethral vaginal muscle layer to the symphysis by the suprapubic approach. Postoperative catheter urethral length measurements confirm the lengthening of the functional urethra. Postoperative cineradiographic studies suggest that the suprapubic approach, particularly in the presence of associated cystocele or prolapse, is less effective in correcting the basic pathophysiologic disturbance associated with stress incontinence.

Our cineradiographic and urethral length studies confirm the ability of the contractions of the levator ani muscles to elevate the bladder base and lengthen the functional urethra, thereby clinically improving states of urinary stress incontinence.[18]

## Summary

1. The total urethral length in the standing position as determined by cineradiography and direct catheter measurement was found to be a reliable index of the degree of female urinary continence.

2. Urinary continence was associated with a total urethral length of 3 cm. or more, indicating competency of the intrinsic musculature and connective tissue and the extrinsic support of the bladder neck and proximal urethra.

3. Urinary stress incontinence was associated with a functionally shortened urethra due to incompetency of the bladder neck and proximal urethra.

4. Urethrocele, cystocele, and procidentia had no direct relationship with the competency or incompetency of the bladder neck and proximal urethra.

5. Successful surgical correction of female stress incontinence restored the competency of the bladder neck and proximal urethra ending in lengthening of the functional urethra.

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## Discussion

Dr. Curtis J. Lund, Rochester, New York. Dr. Gardiner, you are to be congratulated upon the very interesting and timely study which you have so vividly brought to our attention.

Recently, renewed attention has been focussed upon the significance of urethral length in the maintenance of urinary continence. The concept that urethral length is related to urinary continence is not new. For years an accepted surgical principle for the correction of stress incontinence has emphasized the need for lengthening the urethra. Cinefluorography is a tool ideally suited for a study of this type. The use of the beaded chain to measure the length of the urethra is simple and accurate. The method also permits the accurate measurement of the functional urethra as well as the unimportant distal portion which acts as a conduit. Dr. Gardiner has also measured total urethral length by the use of a calibrated catheter in the method of Lapides. Measurements made in the erect position show that, as the average urethral length decreases, the severity of urinary incontinence increases. It is important to note the overlap of individual measurements in all three degrees of stress incontinence. For example, one patient was severely incontinent with a urethral length of 2.5 cm., another was moderately incontinent with a length of 3.7 cm., and still another was continent with a length of 2.5 cm. A comparison between the functional length of the urethra measured radiologically and the catheter technique might be helpful in the explanation of the above discrepancies.

The radiologic measurements were made by the bead technique with the bladder filled. This does not necessarily indicate the length of the resting urethra when the bladder is empty. If the urethra shortens as it fills, then the shortening must be due to change in tonicity, and length then becomes a secondary function of this tonicity and elasticity. This loss of tone permits the development of the characteristic funnel deformity which is naturally associated with a functional urethral shortening. However, not all stress incontinence is associated with a shortened urethra or a funnel deformity. We have observed a few patients with postoperative stress incontinence who appeared to have a urethra of normal length. These patients have been successfully treated with urethral suspension by suture or sling.

There is general agreement that the myth of the internal sphincter has been exploded. Little mention has been made of the voluntary muscles of the urogenital diaphragm which act as a sphincter in the fixed portion of the urethra. Although this muscle is not absolutely necessary for the maintenance of continence, it is of value as a second line of defense against the loss of urine when small quantities enter the proximal urethra.

Several years ago, we discarded the use of a small mushroom catheter to locate the point at which to begin urethral plication, because the catheter might be pulled down into a dilated, lax urethrovesical segment. If this were the case, urethral plication would begin below the deformity and could result in surgical failure. Dr. Gardiner has given us a very simple and useful tool, the calibrated catheter with a 5 ml. bag. Use of this will readily establish the 4.5 to 5 cm. point at which urethral plication should begin. I shall immediately try this technique in the operating room.

Dr. Conrad G. Collins, New Orleans, Louisiana. I enjoyed Dr. Gardiner's paper and also Dr. Lund's discussion. I believe that the length of the urethra does not have anything to do with incontinence because many times it has been necessary to remove up to four fifths of the urethra to get widely around a carcinomatous growth of the vulva and the patient is still continent. This is accomplished by the old method of plicating the fascia and suspending the urethra high. The functioning area that has been described is very important. High fixation of urethra with sutures and getting the urethra back to normal or almost normal caliber are important to maintain these cases, even though the distal portion has been removed by operation.

Dr. C. Paul Hodgkinson, Detroit, Michigan. I congratulate Dr. Gardiner on this excellent study. He is on the way to making a real contribution to our better understanding of urinary incontinence and urinary continence. I need not remind you that we really do not know the true physiologic mechanism which is involved in urinary continence, and until this is known any theory of incontinence is likely to be somewhat erroneous. This problem of urethral length and decompensation of the urethra, although attractive, is difficult to evaluate. I remind you that Lapides did his work on highly dissected dogs.

He successively amputated the urethra at 1 cm. intervals until he found the point where the bladder tended to be incontinent. The critical point was around 3 to 4 cm., which is about the length of the average female urethra.

It must be recognized that he shortened the urethra from the distal and not from the proximal end, which is the point with which we are concerned. I think it is premature to transpose Lapides' studies to clinical practice.

I recently read a monograph by Goran Enhorning (Acta chir scandinav., suppl. 276, 1961), an investigator who has done simultaneous urethrovesical pressure studies similar to those previously reported by us, who feels that urethral length has nothing to do with urinary continence. I am inclined to agree with this opinion.

Francis Bacon said it is a peculiar error of human intellect to be more moved by affirmatives than by negatives, but in the establishment of any axiom, the negative is the more forcible of the two. I am sure it is true that the urethra can be amputated from the distal end close to the bladder and not have urinary incontinence. I recently saw in consultation a 13-year-old girl who had a urogenital sinus and no vagina. On pelvic examination the fingers entered the bladder directly through this open sinus. It was likely she had been having intercourse through this orifice; yet she was not incontinent. It is very confusing to try to reconcile this unusual physical state with any theory of urinary continence.

Again I want to congratulate Dr. Gardiner on his excellent study and I urge him to continue.

DR. GARDINER (Closing). I should like to thank the discussants. As to the patient with malignancy, who loses most of the proximal urethra, we have not had a chance to study such a case. My feeling is, however, that good intrinsic tone of the bladder neck and upper urethra can maintain continence even though most of the urethra has been lost.

Again I want to express my appreciation for the opportunity and privilege of making this presentation.

# Endoscopic studies of esophagus and stomach during pregnancy

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DURING pregnancy symptoms referable to the upper gastrointestinal tract are common. Although extensive investigations of the esophagus and stomach have been reported in nonpregnant individuals, very little fundamental study has been carried out during gestation. The literature contains references to studies of gastric contents,[5, 9, 12, 14, 22, 25] roentgenologic changes,[7, 8, 20] unusual case reports of hemorrhage[6, 13] or peptic ulcer,[2, 4, 18] and a few retrospective analyses,[21] but no evidence was found of a definitive study based upon the endoscopic procedures of esophagoscopy and gastroscopy in pregnant women.

Although gastroscopy had been occasionally practiced since the early attempts of Kussmaul[19] in 1868, the first practical diagnostic instrument came into being with the introduction of the flexible gastroscope[19] in 1932 by Wolf, an optical physicist, and Rudolph Schindler, a gastroenterologist. Esophagoscopy was known as a comparatively unsatisfactory and dangerous maneuver except in the hands of a few experts, such as Chevalier Jackson and his pupils. However, when Boros[3] reported the development of a flexible esophagoscope in 1947, the area of esophagoscopy as well as gastroscopy became the forte of the gastroenterologist as never before.

Today, flexible esophagoscopy and gastroscopy examination is a safe and not overly uncomfortable outpatient procedure; it is accomplished with facility and ease.[1, 10, 11, 16, 19]

An investigation utilizing the latest techniques of endoscopy was undertaken during pregnancy because of the frequency of significant symptomatology and the paucity of fundamental knowledge of the upper gastrointestinal tract during gestation.

## Material

A preliminary investigation of esophagoscopy and gastroscopy was carried out in a group of 15 pregnant women from 5 to 9 months' gestation (Table I). Eleven had upper gastrointestinal symptoms and 2 of these were restudied in the postpartum period. Four asymptomatic individuals were used as controls. The ages ranged from 17 to 40 years. Two were pregnant for the first time and the rest were multigravidas with parity of from 1 to 11.

The most common symptoms were heartburn, epigastric or substernal distress, dysphagia, and varying degrees of belching, nausea, and vomiting. Three patients exhibited hematemesis.

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**Table I**

| *Case No.* | *Initials* | *Age* | *Gravidity* | *Parity* | *Estimated date of confinement* | *Date of endoscopy* | *Period of gestation* | *Past history of gastrointestinal symptoms* | *Severity of symptoms* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | E. H. | 37 | xi | x | 4/28/60 | 1/26/60 | 6th month | Heartburn with each pregnancy subsiding between pregnancies: no history of peptic ulcer | 4+ |
| 2 | D. S. | 36 | ix | viii | 5/27/60 | 5/19/60 | 9th month | Heartburn during past 3 pregnancies; asymptomatic between pregnancies | 4+ |
| 3 | I. L. | 24 | ii | i | 6/ 6/60 | 5/26/60 | 8th month | Heartburn with first pregnancy; no previous symptoms of GI distress | 2+ |
| 4 | G. G.† | 32 | vii | iv | 8/ 4/60 | 5/26/60 | 7th month | Asymptomatic (normal); control | 0 |
| 5 | G. J. | 18 | iv | iii | 7/26/60 | 6/ 9/60 | 8th month | Heartburn with all pregnancies; asymptomatic between | 4+ |
| 6 | H. H. | 33 | vii | vi | 7/22/60 | 6/16/60 | 8th month | Heartburn with other pregnancies asymptomatic between | 4+ |
| 7 | P. E. | 27 | ix | viii | 9/19/60 | 7/ 4/60 | 7th month | Severe heartburn during pregnancies asymptomatic between | 4+ |
| 8 | E. H.* | 37 | xi | xi | —— | 7/ 7/60 | 10 weeks post partum | Mild heartburn since delivery | 1+ |
| 9 | A. D.† | 17 | i | 0 | 9/28/60 | 9/ 1/60 | 9th month | Postlaryngeal burning (not true heartburn); control | 0 |
| 10 | A. T.† | 19 | i | 0 | 9/25/60 | 9/15/60 | 9th month | Asymptomatic; control | 0 |
| 11 | G. B.† | 33 | iv | ii | 10/ 5/60 | 9/29/60 | 9th month | Postpharyngeal burning; not considered to have true heartburn; control | 0 |
| 12 | T. W. | 31 | iv | ii | 11/17/60 | 9/29/60 | 8th month | No past history of heartburn or epigastric distress; mild heartburn with present pregnancy | 1+ |
| 13 | L. Q. | 19 | ii | i | 4/ 8/61 | 10/20/60 | 5th month | Patient hospitalized with severe hyperemesis gravidarum with hematemesis and mild heartburn; no past history of GI symptoms | 1+ |
| 14 | E. F. | 24 | vii | viii | 3/26/61 | 1/29/61 | 7th month | Severe heartburn during all previous pregnancies; asymptomatic between; patient hospitalized on 1/23/61 with severe substernal and epigastric distress, nausea, vomiting, and dehydration; hematemesis also present | 4+ |

| *Severity of symptoms* | *Findings on endoscopy* | *Delivery* | *Postpartum course* | *Gastrointestinal follow-up* |
|---|---|---|---|---|
| 4+ | Severe peptic esophagitis with edematous, erythematous, hemorrhagic mucosa involving cardiac end of stomach and lower 3 inches of esophagus (stool guaiac 3+) | O. A.; spontaneous assisted; 5/2/60 | Uneventful | *See No. 8 |
| 4+ | Inflammation and edema of cardiac end of stomach and lower ½ inch of esophagus, mild | O. A.; spontaneous assisted; 5/27/60 | Uneventful | Patient asymptomatic; no follow-up |
| 2+ | Severe erythema, mild edema of lower 2 to 3 inches of esophagus; stomach negative | O. A.; spontaneous assisted; 5/28/60 | Uneventful | Patient asymptomatic; no follow-up |
| 0 | Esophagus and stomach normal | Low cervical cesarean section (chronic hypertension; fetal distress); 7/7/60 | Pyelonephritis | Normal |
| 4+ | Mild inflammation of cardiac end of stomach; severe inflammation of lower 1 inch of esophagus with one large superficial eroded area | O. A.; spontaneous assisted; 8/7/60 | Uneventful | Asymptomatic |
| 4+ | Mild patchy gastritis, cardiac end of stomach; severe bleeding esophagitis of lower 1 inch of esophagus | O. A.; low forceps; 7/21/60 | Uneventful | Asymptomatic |
| 4+ | Large area of edematous, inflamed mucosa with multiple pinpoint hemorrhagic areas in cardiac end of stomach; patchy moderate peptic esophagitis involving lower third of esophagus | L. O. A. - O. A.; low forceps; 8/24/60 | Uneventful | Asymptomatic |
| 1+ | Stomach negative (normal); minimal injection of lower 2 inches of esophagus | | | Upper GI series and barium enema normal; stool guaiac negative |
| 0 | Gastroesophagoscopy normal | R. O. A.; low forceps; 9/28/60 | Uneventful | Asymptomatic |
| 0 | Gastroesophagoscopy normal | O. A.; low forceps; 9/18/60 | Uneventful | Asymptomatic |
| 0 | Gastroesophagoscopy normal | R. O. T. - O. A.; spontaneous assisted; 10/4/60 | Uneventful | Asymptomatic |
| 1+ | Mild esophagitis involving lower half of esophagus | O. A.; spontaneous assisted | Uneventful | Asymptomatic |
| 1+ | Stomach normal; mild edema and erythema of lower 1 inch of esophagus | O. A.; spontaneous assisted; premature infant; 5/6/61 | Schizophrenic reaction | |
| 4+ | Severe inflammation and superficial ulceration of lower third of esophagus and hiatus hernia found; biopsy obtained (chronic inflammation of squamous mucosal fragment) | R. O. A. - O. A.; spontaneous assisted | Symptoms much improved (mild) after treatment and delivery | *See No. 15 |

Table I—Cont'd

| Case No. | Initials | Age | Gravidity | Parity | Estimated date of confinement | Date of endoscopy | Period of gestation | Past history of gastrointestinal symptoms | Severity of symptoms |
|---|---|---|---|---|---|---|---|---|---|
| 15 | E. F.* | 24 | viii | viii | —— | 2/24/61 | 1 week postpartum | Essentially asymptomatic postpartum | 0 |
| 16 | O. K. | 32 | iii | v | 3/10/61 | 3/ 3/61 | 9th month | No previous history of epigastric distress or heartburn; symptoms present one month prior to gastroscopy | 2+ |
| 17 | G. T. | 40 | xi | iv | 5/30/61 | 5/19/61 | 9th month | Severe heartburn commencing 4th month; no previous history of epigastric distress with pregnancy or between pregnancies; several months of antiacid therapy before endoscopic study | 2+ |

*Repeat endosopic examination post partum.
†Control.

Seven of the 11 women who presented with severe symptoms had significant gastrointestinal symptoms with previous pregnancies. Between pregnancies these symptoms were mild or disappeared altogether. Of the 4 patients without positive past history 3 had the mildest symptoms in the group. The fourth (G. T.) had rather marked heartburn at the fourth month which was partially alleviated by the routine therapy outlined in this report.

## Method

Each patient was given meperidine, 100 mg., and atropine sulfate, 0.4 mg., hypodermically. In approximately 30 minutes the pharynx was sprayed with 2 per cent solution of tetracaine until the gag reflex was absent. The patient was then placed on the left side, the esophagoscope introduced, and the entire esophagus visualized. The flexible gastroscope was routinely passed through the esophagoscope and the cardiac end of the stomach viewed. This single intubation technique was well tolerated by all patients, there being no untoward reactions in either mother or fetus.

The Eder-Huffner esophagoscope and flexible gastroscope were used in all studies. Biopsies were taken on some patients. Fig. 1 illustrates normal esophagus. Fig. 2 depicts acute esophagitis.

Photographs of the lower esophagus were obtained with the Brubaker Endoscopic Camera. Fig. 3 shows moderate esophageal injection, Fig. 4 moderately severe esophagitis, Fig. 5 mild inflammation of the gastric antrum, and Fig. 6 edema and hyperemia of esophagus.

The patients with severe symptoms were asked to follow a bland diet, with small feedings four to five times daily. Medication with magnesium-aluminum hydroxide (Maalox) or oxethazine in alumina gel (Oxaine) in 2 dram doses was prescribed every hour between meals and at bedtime. It was noted that some elevation of the upper portion of the body in bed seemed to bring definite relief of symptoms.

## Results

The four asymptomatic control patients showed no inflammatory change in the lower esophagus and none had demonstrable pathology in the stomach (Table I).

The 11 individuals with upper gastrointestinal symptoms all showed changes except one (O. K.), whose mild symptoms had been present for only one month and who never before had had gastrointestinal dis-

| *Severity of symptoms* | *Findings on endoscopy* | *Delivery* | *Postpartum course* | *Gastrointestinal follow-up* |
|---|---|---|---|---|
| 0 | Mild gastritis in area of hiatus hernia; upper GI series, stool guaiac negative | | Uneventful | Asymptomatic |
| 2+ | Stomach and esophagus normal at time of examination (only patient with symptoms of true heartburn who had normal findings on gastroesophagoscopy) | O. A.; spontaneous assisted; 3/4/61 | Uneventful | Asymptomatic |
| 2+ | Lower portion of esophagus reddened, without ulceration; mild esophagitis | Twin No. 1, R. O. A. - O. A., low forceps; Twin No. 2, internal podalic version and extraction; 5/20/61 | Uneventful | Asymptomatic |

tress. Of the other 10, 5 had marked pathologic conditions, 2 had moderate changes, and 3 had only mild edema and erythema of the lower portion of the esophagus.

Those with marked pathologic conditions including ulceration, edema, and severe inflammation had the most pronounced symptoms, as a rule. Two of these had hematemesis and one had an associated hiatus hernia. All had some involvement of the mucosa at the cardiac end of the stomach. The 2 with moderate symptoms (D. S. and I. L.) had moderate to severe nonulcerative erythema of the lower esophagus. Two of the 3 with mild esophagitis had negative past histories and experienced comparatively minimal epigastric distress and heartburn. (G. T. had received therapy since the fifth month of gestation.) The third had severe hyperemesis gravidarum and, on one occasion, vomited blood; only mild heartburn was present.

Two of the patients with severe changes and marked symptoms were also examined with the endoscope in the postpartum period. One (E. H.) still had slight heartburn and very mild injection of the lower 2 inches of the esophagus. The stomach was normal. The second (E. F.) was restudied only one week post partum and had had no significant amount of treatment. She became asymptomatic immediately after delivery and the esophageal ulceration had healed. There was only mild gastritis present in the area of hiatus hernia.

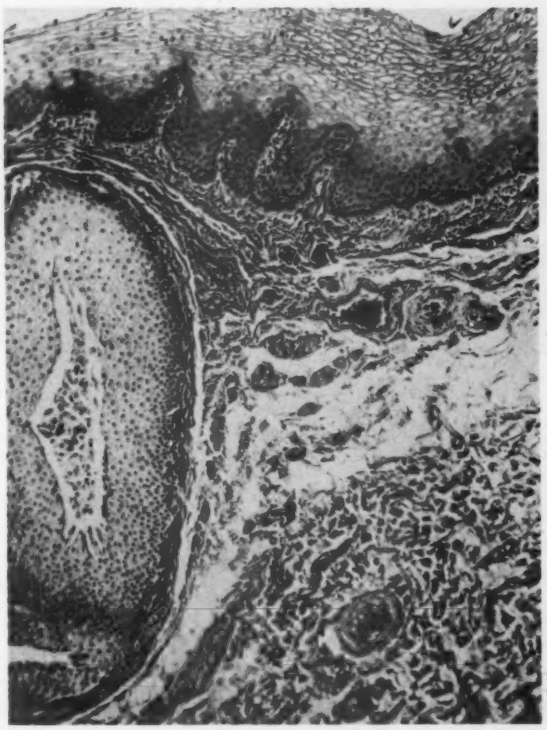

**Fig. 1.** Mucosa and submucosal area of normal esophagus with glandular collecting duct. (Hematoxylin and eosin. ×120; reduced ⅔.)

Although medical therapy as outlined brought about symptomatic relief in a number of patients, it was dramatically evident that the most pronounced improvement usually followed delivery.

### Comment

This preliminary study strongly suggests that visible pathologic changes occur in a very high percentage of pregnant patients who have heartburn and related upper gastrointestinal symptoms. The severity of these changes in the lower esophagus and cardiac end of the stomach has been made surprisingly evident by this esophagogastroscopic study. Work carried out in the past by indirect means,[23, 26, 27] such as x-ray, has shown some evidence of gastric regurgitation into the esophagus as well as occasional lower esophageal spasm and suggestive changes in mucosal pattern. With the more direct approach of endoscopy, the changes are dramatized and it makes more logical the reports[5, 13, 18, 21, 24] which suggest that serious chronic disease of this area may indeed have its beginning during gestation and be exacerbated by repeated pregnancies.

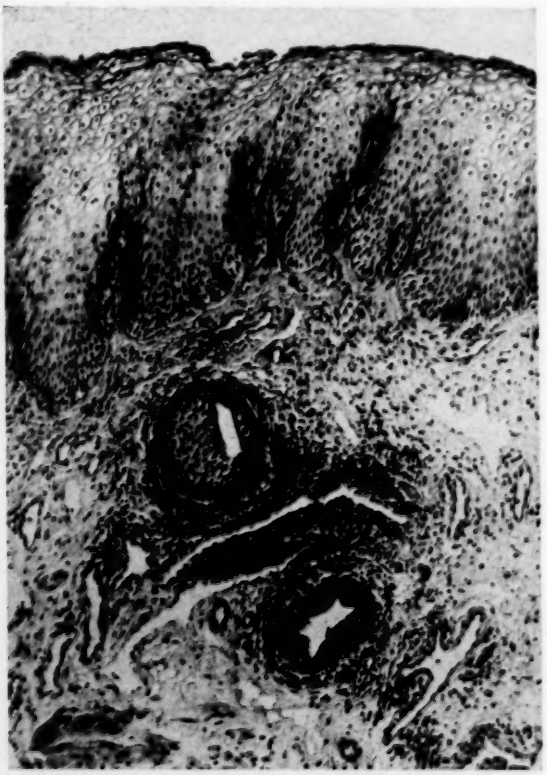

**Fig. 2. Mucosal area of esophagus with acute and chronic inflammation. Note scattered lymphocytes and polymorphonuclear leukocytes about glandular collecting ducts and blood vessels. (Hematoxylin and eosin. ×120; reduced ⅔.)**

Scott and Deutsch[21] have reported a number of cases demonstrating serious pathologic conditions of the lower esophagus such as cicatrization and hemorrhage which seemed to have advanced insidiously through the years after the pregnancies in which the original gastrointestinal symptoms appeared. The present investigation has emphasized the fact that most patients with severe anatomic changes have had repeated and recurrent similar difficulties in past pregnancies. In most instances, symptoms subside markedly with the termination of pregnancy. Furthermore, as shown in 2 of our patients with advanced esophagitis, the anatomic evidence of disease also subsided in an almost miraculous manner.

Although the literature contains conflicting reports as to the production of hydrochloric acid during pregnancy, it now seems fairly certain that during the first two thirds of the pregnancy there is very little deviation from normal, while in the last trimester this is certainly rather markedly elevated.[9] Spiro and his associates[22] were among the first to record in detail a series of gastric aspirations in an ulcer patient during pregnancy. They felt that there was definite correlation between emotional states and gastric secretory levels. Vasicka and associates[24] feel that the elevation of 17-hydroxysteroids (four times normal nonpregnancy levels) tends to aggravate peptic ulcer formation or complications associated with pre-existing ulcer. Such hormonal factors, plus the knowledge that in the latter half of pregnancy there is a tendency toward regurgitation of gastric contents through a relaxed cardioesophageal "sphincter" into the lower esophagus, may well account for the common development of heartburn and the pathologic findings associated with it.[1, 27]

Often in the past the common attitude of both gastroenterologist and obstetrician has

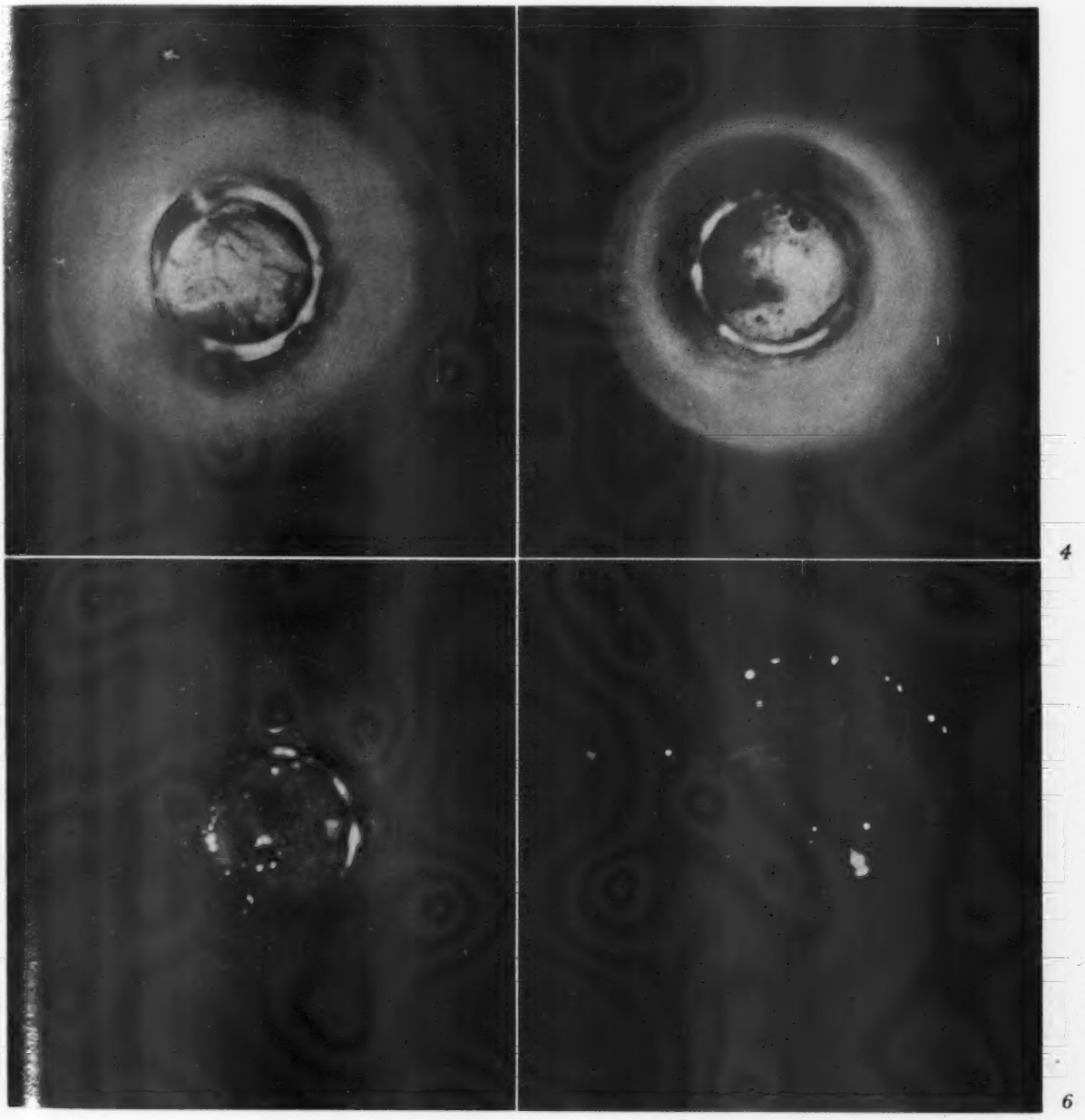

Fig. 3. There is moderate injection of the lower esophagus with dilatation of vessels denoting mild to moderate esophagitis.

Fig. 4. Moderate injection of lower esophagus associated with regurgitated gastric contents.

Fig. 5. Antrum of stomach with mild hyperemia and edema.

Fig. 6. Lower segment of esophagus with edema and hyperemia.

been to avoid thorough diagnostic and therapeutic procedures in these women while decrying the fact that so little is actually known of the physiology and pathophysiology of the upper gastrointestinal tract in the pregnant woman. It is hoped that the present study may serve as a positive step, not only toward abandoning this attitude, but also in stimulating further perceptive investigation in this field.

It would appear that endoscopic studies should be utilized much more frequently during gestation to ascertain the severity of local changes and to serve as a guide to therapy. The subsequent development of chronic lower esophageal disease could thus be averted in many instances. It is impressively evident from this preliminary study that serious anatomic changes do occur quite frequently in the esophagus and stomach during pregnancy. The reports of serious complications, such as massive hemorrhage and perforated ulcer, which are seen in the literature sporadically should give us further impetus to prevent such tragedies by freer use of endoscopy in all pregnant women with significant upper gastrointestinal symptoms.

**Summary**

1. A preliminary report is presented of endoscopic study in pregnant women with common upper gastrointestinal symptoms.

2. Significant esophagitis, sometimes with ulceration, was encountered in the majority of patients. In those with the most marked symptoms there was also involvement of the mucosa of the cardiac end of the stomach.

3. The immediate and remote significance of these changes has been discussed briefly.

4. The importance of utilizing esophagogastroscopy more frequently in the symptomatic pregnant woman is emphasized.

The color illustrations used in this article were obtained through the courtesy and cooperation of Dr. John M. Weber, Dr. Francis C. Jackson, Dr. William Merchant and Messrs. Willis Underwood and Hubert Schwartz of the Oakland Veterans Administration Hospital, Pittsburgh, Pennsylvania.

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## Discussion

Dr. Andrew A. Marchetti, Washington, D. C. The authors are to be commended for bringing to our attention a new approach to the study of persistent gastrointestinal symptoms in the pregnant woman. The endoscopic study of the esophagus and the stomach of these patients is indeed new and unique. A little more unique, is the fact that the discussant volunteered to comment upon a subject with which he was totally unacquainted and hence left him as the saying goes, with a completely unprejudiced mind. However, he was only a short distance away from an authoritative source of help. In March of this year there appeared in the *American Journal of the Medical Sciences* an article by Dr. Irving B. Brick entitled "Esophagoscopy by and for the Internist: A Review of Results in a Thousand Patients." Dr. Brick is a Professor of Medicine and Chief of the Gastroenterology Division at the Georgetown University School of Medicine. He is cited then as the authority and the source to which I turned. After having explained my mission to him, he promptly expressed his willingness to help me with the following statement: "It sounds very interesting, but I know nothing about it."

In this preliminary investigation it is obvious that the authors studied 14 pregnant women to show that their thesis is plausible and that the number of patients would not statistically afford sufficient data to permit of any substantial analysis of their results.

It is currently agreed that, since the introduction of the flexible esophagoscope, endoscopy of the esophagus and the stomach is a relatively safe procedure with little discomfort to the patient. In discussing with Dr. Brick the author's method of premedication of their patients, it was noted that they administer 100 mg. of meperidine hydrochloride and 0.4 mg. of atropine sulfate hypodermically. Dr. Brick stated that for the past 2 years the 100 mg. of meperidine hydrochloride have been given intravenously 5 minutes prior to endoscopy and that this affords very good relaxation and cooperation by the patient. He further stated that he uses 5 ml. of a 2 per cent solution of Pontocaine as a gargle instead of using it as a spray. Whatever method is used, it was pointed out, one should bear in mind the danger of a toxic or allergic reaction to the topical application of cocaine derivatives, especially in those areas where it can be rapidly absorbed.

In the table provided to me there was only one biopsy recorded. It appears that if a more extensive study were to be undertaken in the future, more biopsies should be a desideratum.

The suggestion that, as a result of their preliminary endoscopic studies, visible pathologic changes occur in a very high percentage of pregnant women with heartburn and related upper gastrointestinal symptoms is dramatic cannot be denied. However, that this evidence makes more logical the reports suggesting that serious chronic disease of this area may be initiated by gestation and be exacerbated by repeated pregnancies is difficult to accept at the present time.

The fact that in most instances of these cases the symptoms rapidly subside with the termination of pregnancy and the fact that in 2 of the author's cases the anatomic evidence of severe esophagitis disappeared "in an almost miraculous manner" would imply that there is still much to be done to prove that there is a direct relationship between chronic disease of the esophagus and stomach and the acute gastrointestinal symptoms described in the last trimester of pregnancy.

Nevertheless a new field of perceptive investigation has been opened by Dr. McCall and his co-workers and it is felt that their plea to study more thoroughly the physiology and pathophysiology of the upper gastrointestinal tract in pregnant women by utilizing more frequently the endoscopic approach should be supported.

Dr. McCall (Closing). We are very grateful to Dr. Marchetti for his thoughtful and stimulating discussion. His enthusiasm for delving into the details of the methods described is impressive, and I am especially grateful for his addition of Dr. Brick's work from his own medical center. While the suggestion of administering meperidine hydrochloride intravenously before endoscopy may prove helpful in some instances, we have not been concerned that the moderate degree of sedation given to our patients is ordinarily inadequate.

The subject under discussion is an age-old problem. Just 20 years ago, here at the Broadmoor at a meeting of the American Gynecological Society, Williams read a paper on heartburn in pregnancy. In this study the delayed emptying time of the stomach during pregnancy was emphasized and it was suggested that neostigmine be used to correct this. In recent times more scientific methods have been utilized in the

nonpregnant. Inglefinger has pointed out the pressure differentials in the upper gastrointestinal system. However, there are still many mysteries concerning this system in pregnancy. It seems strange that endoscopy of this type has not been studied specifically in the pregnant woman. We must realize how very broad our specialty of obstetrics and gynecology actually is. It has tremendous medical as well as surgical implications. Dr. Marchetti pointed out quite correctly that our study is preliminary. There are many fascinating studies to be done, and the fact that Dr. Marchetti and his associates plan to use endoscopy makes us hopeful that even such a preliminary investigation as this may indeed prove worth while.

# Psychophysical indications for hymenal dilatation

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IN GREEK mythology, *Hymen* was the name of the god of marriages, and the *hymenaeus* was the song sung at marriage ceremonies. A like term was used to describe the serous membranes, but there is no indication that the Greeks used the word *hymen* in relationship to the vagina. It was not until the time of Vesalius that the word was restricted to its present meaning. In English its use as applied to the vaginal membrane was recorded first in the year 1615.[1]

The hymen, essentially a membranous structure, should rank second in anatomic and physiologic importance to the better developed, more functional levator ani muscles, for it is almost always spasm of the levators which produces the entity known as vaginismus. The levator spasm, when present, may be a variant of frigidity in that the vaginismus usually prevents sexual fulfillment. From the days of folklore to modern times, however, the hymen has been referred to as the primary site of psychosexual discomfort. However, it is only in virgins that dilatation of the intact hymen may contribute to the pain mechanism which is initiated by attempted intercourse.

Through the centuries tribal taboos, religious dogmas, and marital customs have all contributed to the mysticism surrounding the hymen. Our present culture has done little to dispel the superstitions pertaining to this usually innocuous vaginal membrane and to most of the problems involving the sexual organs. Because of that fact, even today, the management of many psychosexual disorders continues to be difficult.

Although the presence of an intact hymen may be of great significance in a virgin bride or in a "married virgin" not enough thought has been devoted to this entity in recent textbooks of gynecology or those of related subjects.[2-5] Relatively few articles pertaining to this entity have been found in the gynecologic literature of the past few years.[6-9] The psychiatric literature has a fund of information concerning frigidity and vaginismus, but other than for a few theoretical observations, it does not deal specificially with the anatomicophysiologic aspects of the hymen.

Premarital counseling and the correction of the less serious disorders of psychosexual function is within the realm of all physicians who treat obstetric and gynecologic patients.[10] Often such physicians fail to appreciate the magnitude of some of the more common psychosexual problems which they encounter.[11] The physician may be too reticent or too embarrassed to discuss such problems with his patients.[6] In turn, it may be difficult or impossible for the patient to communicate her inward feelings to the doctor concerning the basic aspects of her sexual life. A patient with vaginismus may be sent abruptly to a marriage counselor or to a psychiatrist with no active effort having been made to solve the distressing problems which she has presented. The therapy of all but the most profound degrees of disturbed psycho-

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sexual relationships in women should be initiated by the first physician whom the patient consults.[11] The presence of an intact hymen in a premarital patient may or may not be of clinical significance. In the "married virgin," however, the presence of an intact hymen with its accompanying levator spasm is usually indicative of a serious degree of psychosexual disturbance.[10] The successful management of such patients may require the utmost in professional skill.

## Material

This presentation is concerned with three groups of women in whom psychosexual disturbances may be a problem. The three are:

1. This patient consults the physician for premarital counseling. She is usually in her early twenties. An undilated hymen and levator muscle spasm may make pelvic examination difficult if not impossible. Intercourse after marriage in such patients has usually proved to be traumatic both physically and emotionally. At times it has been productive of psychosexual abnormality in later months or years. It is in this group of patients that hymenal dilatation produces its optimum results and may prevent emotional conflicts from arising later. It must be emphasized, however, that only a few premarital virgins require artificial dilatation prior to marriage. The proper management of the group who do need treatment fulfills all of the criteria of excellent preventive medicine.

2. The patient who has been married for weeks, months, or years and who has been unable to have sexual intercourse is typical of this group. The longer that such an abnormal state has persisted, the more difficult may be its treatment.[12] An insignificant number of these women will have seen a physician prior to marriage, but many will have done so after the marital relationship had proved to be unsuccessful. Many will have been dismissed with the thought that "everything will be all right, see me again in six months." An inadequate history will fail to reveal the true nature of the patient's problem for few women volunteer information relating to their sexual lives. Many patients with psychosexual problems will present only vague psychosomatic complaints to the physician.[11] Much psychosexual pathology will remain undetected in the absence of polite but persistent inquiry. The gynecologist may collaborate unwittingly with the patient to keep undetected the real reason for her visit. This represents an "unconscious conspiracy" between the patient and the doctor for the purpose of avoiding certain tension-producing discussions which might be unpleasant for both. It represents a polite evasion of the truth for although the truth would help its exposure might hurt.

In many instances, in addition to the inadequate history, no pelvic examination will have been done, presumably on the basis that "time will take care of everything."

3. In this group of patients, intromission has been rendered difficult if not impossible by obstetric or gynecologic procedures subsequent to marriage. Only the constant awareness of this as an end result of obstetric and gynecologic operations will prevent this iatrogenic entity.[13]

## Methods

In all three groups of patients, excellent rapport must be established if the physician is to become fully informed concerning all of the ramifications of the patient's problems. With the premarital group, it is better in most instances to obtain only a brief initial history. The pelvic examination which follows will supply the answers to many of the pertinent questions which may have been tactfully omitted by the physician. In the majority of married patients in whom intromission has not occurred, the patients may be too embarrassed to tell the physician the real reason for their consultations. In this series, none of the "married virgins" spontaneously offered the complaint that intromission had been impossible of attainment.

When a premarital or a married patient is encountered who has an undilated hymen the physician should not persist in trying to examine the patient vaginally. An explanation should be given to her concerning the physi-

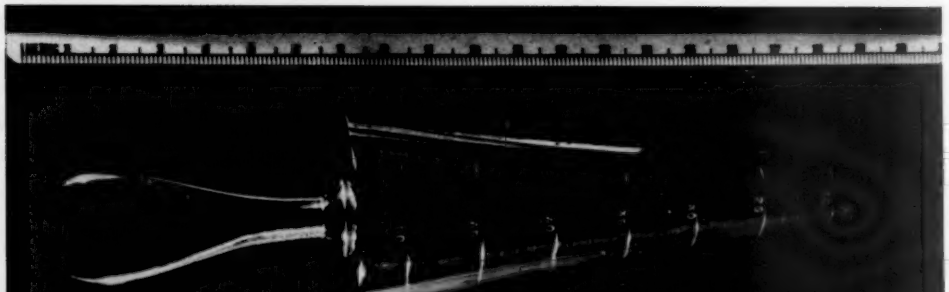

**Fig. 1. The graduated Kelly orifice dilator.**

cal problem involved. A graduated orifice dilator (Fig. 1) is given to the patient. The physician should show the patient how to introduce the dilator into the vagina, but he should make no effort to stretch the hymenal ring. She is instructed to use the dilator for a 15 to 20 minute period once or twice daily. She is asked to return in 2 weeks for a follow-up visit. This therapy has proved to be so satisfactory that no operative procedures have been necessary in this group of patients for the past 10 years.

The dilatation should always be carried out with an explanation to and with the complete concurrence of the patient's fiancé. It is important that he understand that although the eventual intercourse will be physically painless she is still virginal, both morally and legally. By the fiancé's concurrence, misgivings and misunderstanding will be avoided.

Through the use of the graduated dilator, this group of patients will be able to actively stretch the hymen and to reduce the levator muscle spasm that is present. Subsequent pelvic examination and ultimate intercourse will then be possible. There may be an occasional patient in whom an additional week or 10 days will be necessary before complete hymenal dilatation can be achieved. If the patient is of the "married virgin" type she is told to make no further attempts to have intercourse until dilatation has been adjudged successful by the physician.

One of the advantages of the graduated dilator is that the patient does not have to change from an instrument of smaller size to that of larger size. The latter is the case when glass tubes of increasing caliber are used. Then too, many patients are unsuccessful in using the graduated tubes because of the fear of introducing glass into the vagina. The latter may reinforce in some women their unconscious childhood fears pertaining to genital injury. The graduated tubes also present the patient with the needless necessity of dealing with multiple dilators and the need to progress by definite steps. Still another factor is that patients are able to rationalize failure to progress from one tube to the next. All of the above may introduce additional psychological problems of major degree. For these reasons the Kelly dilator has been found to be more satisfactory than the glass dilators upon which some previous reports were based.[6, 7, 12, 14]

After the patient has been able to introduce the orifice dilator up to the 45 mm. or 50 mm. level she can be told truthfully that she will have no physical difficulty in having intercourse and that she is now "large enough." It is important that this regimen of therapy should be carried out in its entirety by the patient.[15] No attempt should be made by the physician at any time to dilate the hymenal ring. The patient may develop mixed feelings toward the physician who manually or surgically effects her defloration. He may unwittingly complicate his relationship with the patient by such an act. When that happens,

the patient usually will have increasing ambivalence toward the physician.

In the rare instance in which the physician thinks that he must assume an active role in the defloration, it is advisable to consider thoroughly the aforementioned psychological aspects before any intervention is effected. If the need for active defloration ever should arise it is better to delay the operation until psychiatric clearance can be obtained.

The following case reports are presented to illustrate each of three different types of patients seen in this series.

**Premarital patients with an intact hymen.**

*Case 1.* Miss E. M., a 23-year-old patient, was first seen in February, 1955, for premarital examination. The hymenal ring was intact. The patient had a marked degree of levator spasm. The patient was given an orifice dilator to use and within a matter of 2 weeks she was able to dilate the introitus adequately. The patient has reported that her marital and sexual life have been satisfactory. She has had two successful pregnancies subsequently.

*Case 2.* Miss G. C., a 28-year-old patient, was first seen in November, 1956, upon her return from abroad. She had been operated upon in Holland in 1954 for endometriosis. Her principal complaint at the time of her first visit was that of severe dysmenorrhea. Pelvic examination at that time revealed that the introitus would not admit an examining finger.

This patient was given an orifice dilator premaritally in September, 1957. Within 3 weeks she had adequately dilated the hymen and had overcome the levator spasm which had been present. This patient subsequently became pregnant and was delivered of her first baby in August, 1960.

*Case 3.* Miss G. M., a 29-year-old patient, was first seen in February, 1961, for premarital examination. The patient was rather shy. Like the 2 previous patients she had had no sexual contact.

Pelvic examination revealed a completely intact hymenal ring and marked levator spasm. Over a 6 weeks' period this patient was able to slowly but successfully dilate the hymen. After her marriage in April, 1961, intercourse was satisfactory from its inception.

The above are examples of patients who have had self-treatment premaritally by means of an orifice dilator. All of these patients had had no previous sexual experience. Pelvic examination has been significant by virtue of the intact hymen and marked levator spasm. Re-emphasis is made of the fact that few premarital patients, even though they are actually virginal, will require such therapy.

**"Married virgins."**

*Case 1.* Mrs. M. J. P. was first seen in March, 1954. She stated that she had been married for 8 months but that attempts at intercourse had been unsuccessful. Shortly after the initial unsatisfactory attempts she had consulted a physician. He had told her that such was "perfectly normal" and that she and her husband should "just keep on trying." Nothing was suggested in the way of therapy. After a careful history and gentle examination this patient was given an orifice dilator. Within 2 weeks she had succeeded in dilating the introitus. She then became able to participate in sexual relations with her husband to their mutual satisfaction. This patient now is happily married and has 2 children.

*Case 2.* Mrs. N. A., a 28-year-old patient, was first seen in January, 1952, at which time she had been married for 3 years without having had intromission. Shortly after her marriage she had consulted a physician. She stated that she was hurt so much by his attempted examination that she never returned to him. She was afraid to consult anyone else for almost 3 years. After a detailed interview and gentle examination the patient was given an orifice dilator. Within 2 weeks she had dilated the introitus. Three weeks after the patient had been seen for the first time, she and her husband were able to have intercourse successfully after 3 years of married virginity. She has had two babies vaginally.

*Case 3.* Mrs. M. B., a 38-year-old patient, was first seen in July, 1959. At that time she had been married for 9 years without having had sexual intercourse. She had seen physicians on three occasions but in each instance she had been told that she needed to be "cut." The thought of that procedure frightened her to the extent that she had never undergone the proposed operations. When she was seen in 1959 the introitus barely admitted the tip of one finger. Marked levator spasm was present. Instead of advocating an operation, we reassured the patient and gave her an orifice dilator. Within a matter of 4 weeks the patient had succeeded in dilating the introitus. Marital rela-

tions became possible for the first time in 9 years. The patient became pregnant in May, 1960, but unfortunately she had a spontaneous abortion. She has had no further pregnancies although her marital life continues to be satisfactory.

The 3 patients outlined above are fairly typical of a large number of patients in whom intercourse over a matter of months or years had never been consummated. There are many such patients in this group who had never told a physician of their difficulties in spite of repeated visits for the investigation and treatment of various psychosomatic complaints. Many of these patients will have had a painful pelvic examination previously which precluded subsequent examination by a different physician for months or years. Several of the patients had had hymenotomies or perineotomies. The customary surgical procedures had failed in these patients to correct the difficulty.

At first glance, the above patients would appear to have had such severe degrees of psychopathology that the prognosis would have to be considered as quite poor. Detailed interviewing, reassurance, explanation, and the use of the orifice dilator succeeded where other methods had failed.

**The patient with postsurgical stenosis of the introitus.**

*Case 1.* Mrs. R. M., a 58-year-old married patient, had had an anterior and posterior colporrhaphy at the age of 46. She was first seen in July, 1960. Her history revealed that she had not had intercourse since the pelvic operation 12 years previously. Pelvic examination revealed the presence of an introitus which admitted only one finger. This patient was given an orifice dilator. After 4 weeks of dilatation and the use of estrogen creme, the introitus readily admitted two fingers. The patient stated that intercourse had again become possible.

Many patients after obstetric or gynecologic procedures may have a compromise of the area available at the introitus. Dilatation with an orifice dilator will usually be effective and will make further operations unnecessary.

## Comment

In those few premarital patients in whom an intact hymen combined with levator spasm would make initial intromission painful and difficult, the use of an orifice dilator will allow the patient to have intercourse successfully. This simple therapeutic endeavor may prevent much marital unhappiness. It is extremely important, however, that the dilatation be carried out only by the patient. Participation by the patient in her own defloration constitutes active therapy on her part. Conversely, dilatation performed by other than the patient should be designated as passive therapy. It should also be noted that passive therapy may serve to increase the patient's fantasies, thus complicating the relationship with her physician.

Only by active participation in her own treatment will the patient be able to nullify and to overcome her lifelong sexual inhibitions. The latter are the most significant contributors to the levator spasm which the patient has manifested. Active therapy, carried out in the patient's home, in privacy and under favorable circumstances, allows the patient herself to determine the degree and duration of dilatation. The latter is of inestimable value if the therapy is to be completely effective.

In married patients who have not had successful intromission, an orifice dilator will stretch the hymenal ring sufficiently and will help to overcome the levator spasm which has prevented penetration. In general, the married patient who has remained a virgin is more difficult to treat than is the premarital patient, providing that the latter has not had a previous traumatic examination. The so-called "married virgin" usually has vastly significant emotional problems which have contributed markedly to her persistent virginity. The range of her emotional problems may vary from her own conflicts regarding pregnancy to conflicts concerning, among other things, the potency of her husband. In these women, the emotional conflict has been converted into a physical entity, namely, involuntary levator muscle spasm. Parenthetically, tense levator spasm can rarely if ever

be on a voluntary basis,[15] an observation which is consistent with our knowledge of the unconscious forces within any individual. Where the patient is subjected to passive therapy, she may react with an aggravation of the psychic and somatic symptoms inasmuch as she no longer has her previously well-guarded physical disability, namely, the unpenetrated introitus. With the physical aspect of the problem having been eliminated, the underlying psychosexual condition may become intensified unless the patient has other defensive mechanisms to replace the unconscious usefulness of the previously intact vaginal orifice. In psychiatric terminology, this is described as the "ego-defensive" mechanism. Some patients following surgical defloration have attempted suicide.[12]

By contrast, active therapy is completely under the control of the patient. If the patient rejects or is only partially successful with the dilator therapy, the likelihood is strong that fairly deep-seated psychosexual problems are present. Such patients will usually require psychiatric consultation.

In the post-surgical group, the factor of true physical compromise of the introitus is the dominant factor initially. By virtue of repeated attempts at intromission, however, most of these patients will have developed some degree of superimposed psychogenic muscle spasm.

Surgery in the form of hymenotomy or perineotomy has had a diminishing role in the management of this condition. Any hymenal or perineal operation leaves a temporarily painful scar at best. Therefore, surgical measures should be avoided in the premarital patient. With the "married virgin," whose problem is further complicated by more serious psychosexual overtones, surgical incision of the hymen may precipitate an acute anxiety state, which is even more important than the painful scar which will be added to the already present spasm. It is in order to add that a surgical attack on a psychogenic entity is seldom successful.[16]

In all of these patients the utmost in gentleness and understanding must prevail during the interview with and the examination of the patient. These patients have many emotional problems and sensitivities. Most of the "married virgins" will have seen several doctors. Painful examinations usually will have intensified the problem from time to time. Physicians who have been unacquainted with an adequate understanding of the depth of this entity unintentionally may have made the problem of the patient an even greater one.

An unsuccessful marital relationship may lead to infertility, to infidelity, to untold psychosomatic problems, to needless gynecologic and other surgical procedures, and to divorce. In the interest of promoting happier marriages, the gynecologist may make a distinct contribution by his careful, considerate investigation of the causes underlying certain sexual difficulties. He may be of great value in guiding the patient into the most beneficial type of therapy for her problem.

## Conclusions

1. The use of an orifice dilator is of inestimable value in hymenal dilatation and in the alleviation of the accompanying levator spasm.

2. Hymenal dilatation in the premarital patient, when indicated, is best carried out actively by the patient.

3. The "married virgin" and the post-surgical patient can similarly be treated most successfully by means of an orifice dilator.

4. In all of these patients, thoughtful recognition of the emotional factors involved is of the greatest importance in the successful management of the patients.

5. Operations on the hymen or the perineum for the prevention or the relief of this problem are rarely necessary.

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## Discussion

Dr. Ralph C. Benson, Portland, Oregon. This most interesting and timely contribution is a partial consideration of gynetresia, dyspareunia vaginismus, and frigidity. These problems are most difficult to manage in practice. The authors properly consider difficulty in intromission to be due to (1) mechanical obstruction, and (2) psychosexually induced muscle spasm.

Sudden stretching of a thick annular hymen may cause pain. Yet, as Drs. Barter and Yochelson say, ". . . it is only in virgins that dilatation of the intact hymen may contribute to the pain mechanism which is initiated by attempted intercourse." Where gynetresia no longer exists, I doubt that the hymen is the "primary site of psychosexual discomfort," as the authors suggest. The hymen is only the gateway to an avenue, forbidden to some, frightening to others. Obviously, symbolism and taboos still relate psychosexual difficulties to the hymen in our culture. Vaginismus and frigidity usually are psychological defense mechanisms, not a trigger-response to pain.

The essayists wisely stress the need for a complete history and pelvic examination by the physician in marriage counseling. Certainly, vaginismus is evidence of a psychosexual aberration *with or without* an intact hymen in the married woman who presents no organic pathology.

Like conversation, the process of interview is an art which can and must be learned. There are techniques which the physician may use to obtain deftly significant background material, even when this is emotionally charged. I especially like the authors' reference to the avoidance of an "unconscious conspiracy" between the tense patient and the harried doctor who both try to shun embarrassing facts.

An imperforate or virtually occlusive hymen demands operation; most other cases, particularly those associated with vaginismus, are best treated medically. I endorse the suggested program of therapy including the clever use of the Kelly dilator. We must remember that surgical attack on a psychogenic entity is seldom successful.

Avoidance of intercourse on a quasiphysical basis may relate to the patient's gender role. Not all marriages are "made in Heaven"; the woman may not actually love her husband. Some marriages are arranged for convenience, others for money. Sexual responsiveness is not guaranteed by the marriage pact. Vaginismus may also arise from a mortifying fear of pregnancy. An explanation and contraceptive advice will often completely dispel refusal of coitus.

Frequently, women are somatically equipped but are psychologically unprepared for marriage. Some are unadjusted, others maladjusted. Basically, the woman must experience an anticipatory emotional excitement as a prelude to sexual arousal. Psychosexual inhibitions prevent this necessary "build-up" so that orgastic satisfaction does not follow. Satisfactory conditioning and sexual fantasy are required for arousal.

Mann[1] has indicated that "some women subconsciously consider the sexual act so threatening that they translate their sexual anxiety into the somatic idiom of vaginismus." Hence, anticipation and participation are prevented.

I am convinced that both authors are psychiatrists in truth. Their plan has been successful in the cure of a sizable number of patients. However, I believe that Drs. Barter and Yochelson are too modest because, in addition to the dilatation of the hymen with the Kelly dilator, they also have helped their patients resolve their fears and inhibitions. The dilator is not a "do-it-yourself kit" or a phallic symbol. It is, however, a token of the doctor's sincere desire

to help. Certainly the treatment of vaginismus (and frigidity) cannot be entirely mechanistic; psychic impediments must be eliminated. The dilatations, the discussions, the explanations help the patient to work out her problem: she thereby becomes receptive, not resistive or submissive. The goal is emotional maturity, an essential to happiness in marriage.

DR. FRANK R. LOCK, Winston-Salem, North Carolina. I could not be more pleased to have a paper on the program than this one. We are so concerned with maternal and perinatal mortality and those situations which threaten the lives of our patients that we do not give enough time and thought to some conditions which cause them great unhappiness. These are serious problems in human relations which represent the basis for many of the functional diseases.

I was particularly pleased that Dr. Barter has de-emphasized surgical procedures and dilatation by the physician. I think his point that psychiatric consultation is needed for only a small segment of the patients is very important. He has offered a challenge to every physician who sees women to make some effort to understand the problems of psychosexual relationships a little better and to help such patients.

The importance of instructing and counseling the male has not been brought out in this discussion. Certainly a well-intending, inexperienced man is likely to create traumatic emotional and physical situations from which the patient may never recover.

I hope that the future programs of this Society will provide a comprehensive literature for all physicians who want to develop skills in this difficult area.

DR. BARTER (Closing). In his discussion, Dr. Benson brought out many of the points which had to be omitted in the interest of brevity. There is no question that there are many, many facets of this problem which remain to be studied and to be solved. Dr. Benson did say that he did not think the hymen was the primary source of pain in intercourse, and we are in agreement with this. In the manuscript the hymen is referred to as the primary site of pain but it is in itself not the primary source of pain. I believe this is a vaginismus type of problem. Very few of our patients were seen by a psychiatrist. The majority were seen by Dr. Parks and myself only and the need for psychiatric consultation was indeed rare. That was our whole purpose in presenting this paper, namely, that this is the type of problem which every doctor should be able to work out with his patients, thus preventing future psychosexual problems which might ultimately require psychiatric help. We should be able to avoid this in almost all instances.

I deeply appreciate Dr. Lock's very kind remarks. It is encouraging to have his support.

# Gynecological operations in 94 patients with intersexuality

## Implications concerning the endocrine theory of sexual differentiation

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THE last decade has witnessed a revolutionary change in the treatment of intersexuality. It has been only slightly longer than a decade since Wilkins announced that cortisone was effective in reversing the inexorable virilization which previously had plagued the genetic female victims of congenital adrenal hyperplasia.[1] This therapeutic tour de force has given significance to the surgical effort to provide for these patients external genitals feminine in function and appearance. During the same era an evaluation of the long-term functional and psychological results of previous efforts to construct masculine genitals for some genetic male hermaphrodites born with inadequate and ambiguous genitals has resulted in the realization that many of these individuals might well have been happier, more useful citizens had they been reared as women.

The net result of this decade of change has provided gynecology with a challenging opportunity to accept for surgical habilitation the vast majority of patients with all forms of intersexuality. For example, Wilkins,[2] in reviewing 242 consecutive cases of intersexuality, found only 20 patients whose external genitals were really suitable for male reconstruction. The majority of these were among the male hermaphrodites, but even among these the majority—29 out of 47—had genitals which were more suitable for feminine than masculine reconstruction.

The main purposes of this communication are to catalog the procedures used and to call attention to the problems encountered among 94 patients operated upon by the senior author in the 8 year period 1953 to 1960, inclusive (Table I). A secondary purpose is to present certain hypotheses which the findings in the 94 patients suggest with respect to the endocrinological theory of sexual differentiation.

### Female hermaphroditism due to congenital adrenal hyperplasia

There were 39 genetic female patients with congenital adrenal hyperplasia, 37 of whom were correctly reared as girls, and two of whom were unfortunately reared as boys (Table II). Laparotomy for exploratory purposes is no longer necessary or desirable in this syndrome for it has been amply demonstrated that ovaries, tubes, uterus, and vagina (except for its orifice) are entirely normal and the difficulty is confined to masculinization of the external genitals. Thirty-two of the 37 patients reared as girls had successful and satisfactory reconstruction of the external genitals according to the technique previously described,[3] or as modified as will be discussed. In 5 patients in the earlier

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years of this series, the original attempt at reconstruction was unsuccessful and had to be limited to clitorectomy because of failure to find the vaginal orifice or because the vagina was, in fact, noncommunicating.

To aid in finding a tiny vaginal orifice the standard surgical procedure may be modified by enlarging the orifice of the urogenital sinus to allow the introduction of a small McCarthy panendoscope in order to probe visibly or blindly with a tiny ureteral catheter in the hope of finding the vaginal orifice (Figs. 1 and 2).

Failing this, as it is extremely desirable for psychological reasons to complete the necessary operative procedure prior to the development of permanent memory, it becomes necessary to resort to laparotomy for positive identification of the vagina from above. This assures that the procedure may be accomplished (Figs. 3 and 4). It has been necessary to employ this maneuver only twice in this particular group of patients, and since the introduction of the ureteral catheter technique there have been no failures to find the vagina from below during the last 3 years.

In all cases of virilizing female hermaphroditism the surgical correction of the external genitals may be satisfactorily accomplished prior to 2½ years of age, and in most cases before this.

In all 5 patients with reconstructions incomplete at the first attempt the condition has subsequently been corrected.

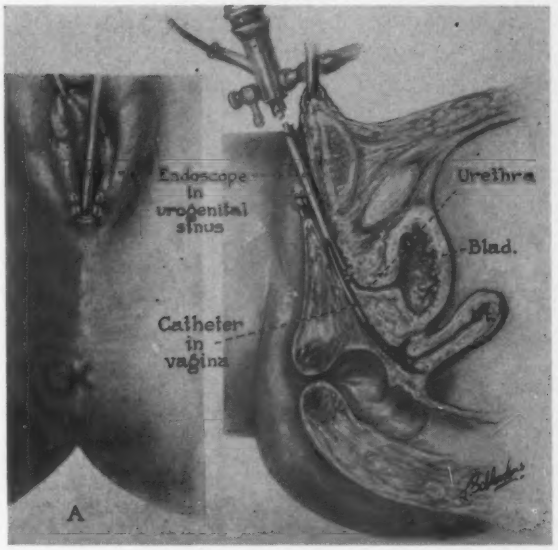


**Fig. 1.** *A*, Enlargement of the orifice of the urogenital sinus to allow the introduction of a catheterizing panendoscope. *B*, Catheterizing panendoscope in the urogenital sinus with the successful catheterization of the vaginal orifice with a tiny ureteral catheter.

The 2 patients incorrectly reared as males were seen at a period in their development when it was considered undesirable to attempt a change in the sex of rearing and for that reason a prepubertal hysterectomy and bilateral salpingo-oophorectomy were carried out to prevent the onset of feminization and menstruation.

### Nonadrenal female hermaphroditism

This small group is composed almost entirely of patients whose mothers' received

**Table I.** Gynecological operative procedures in 94 hermaphrodites

| | *Cases* | *Female rearing* | *Male rearing* | *Laparotomy* | *Clitorectomy* | *Excision of prepuce* | *Exteriorization of vagina* |
|---|---|---|---|---|---|---|---|
| Female hermaphroditism—adrenal | 39 | 37 | 2 | 4 | 36 | 0 | 32 |
| Female hermaphroditism—nonadrenal | 16 | 16 | 0 | 5 | 8 | 6 | 12 |
| Male hermaphroditism—masculinizing | 23 | 21 | 2 | 21 | 20 | 1 | 8 |
| Male hermaphroditism—feminizing | 7 | 7 | 0 | 7 | 1 | 0 | 0 |
| True hermaphroditism | 1 | 1 | 0 | 1 | 1 | 0 | 1 |
| Gonadal dysgenesis | 5 | 4 | 1 | 5 | 2 | 0 | 2 |
| Triple X syndrome | 3 | 3 | 0 | 3 | 0 | 0 | 0 |

**Table II.** Female hermaphroditism due to congenital adrenal hyperplasia

| | | | |
|---|---|---|---|
| Patients with definitive operation reared as females | | | 37 |
| Reconstruction completed | | 32 | |
| Clitorectomy | 31 | | |
| Exteriorization of vaginal orifice | 32 | | |
| Reconstruction incomplete | | 5 | |
| Clitorectomy | 5 | | |
| Incision of urogenital sinus without discovery of vaginal orifice | 5 | | |
| Patients with definitive operation reared as males | | | 2 |
| Hysterectomy, bilateral salpingo-oophorectomy | 2 | | |

**Table III.** Nonadrenal female hermaphroditism

| | | | |
|---|---|---|---|
| Patients with definitive operations reared as females | | | 16 |
| Laparotomy | | 5 | |
| Reconstruction of external genitals | | 15 | |
| Exteriorization of vaginal orifice | 12 | | |
| Excision of redundant prepuce | 6 | | |
| Clitorectomy | 8 | | |

**Table IV.** Male hermaphroditism with partially masculinized genitals

| | | | |
|---|---|---|---|
| Patients with major operations reared as females | | | 21 |
| Laparotomy-gonadectomy | | 21 | |
| External genitals | | 21 | |
| Patients with uterus and vagina | | 5 | |
| Clitorectomy | 5 | | |
| Exteriorization of vagina | 3 | | |
| Further operations necessary | 0 | | |
| Patients with vagina only | | 10 | |
| Clitorectomy | 9 | | |
| Exteriorization of vaginal orifice | 5 | | |
| Lengthening of vagina necessary later | 7 | | |
| Patients without vagina | | 6 | |
| Clitorectomy | 6 | | |
| Construction of artificial vagina later | 6 | | |

**Table V.** Male hermaphroditism with feminization

| | |
|---|---|
| Patients with definitive operation reared as females | 7 |
| Laparotomy with bilateral gonadectomy | 7 |
| Clitorectomy | 1 |
| Vaginal lengthening | 2 |

androgenic steroids during pregnancy. Although only 16 patients required definitive operations (Table III), a much larger group was rejected for operation because the phallus was of insufficient size to warrant excision. In extreme examples the anomaly produced by maternal androgen may be as severe as that in congenital adrenal hyperplasia and the difficulty in locating the vaginal orifice as exasperating. The anatomical details of the anomaly of the external genitals have been previously described and it needs only to be recalled that the internal genitals are quite unaffected by the exogenous androgen.[4] The tendency for the clitoral enlargement to be confined to the fleshy component in this condition is borne out by the fact that reduction in size was accomplished without removing glands or corpora in 6 of the 14 patients in whom the clitoris was disproportionately enlarged (Fig. 5).

It is probably significant that in 1960 only 2 patients with this problem were added to the surgical series, compared with 6 in 1959 and, with the awareness of the undesirable androgenic side effects of some of the progestogens, this particular category of intersexuality will probably become of only historical interest.

### Male hermaphroditism with partially masculinized genitals

The various factors bearing on a decision about the most desirable sex of rearing in patients with intersexuality have been discussed elsewhere and need not be further elaborated.[2, 5] In the newborn the decision to rear a male hermaphrodite as a female is, in large measure, dependent upon the suitability of the external genitals for surgical reconstruction. Borderline cases in the present series have been reviewed with a urologist, Dr. William W. Scott, and if it has seemed possible to construct functional male genitals, the child was so oriented. As a matter of experience it has been decided that about two thirds of all male hermaphrodites seen were better reared as girls. The present discussion chiefly concerns 21 male hermaphrodites who were reared as females. In

addition the series includes 2 reared as males (Table IV).

An important estimation in all male hermaphrodites is whether the malformed testes will produce a biological preponderance of estrogen or androgen at puberty. Feminization may be expected if the genitals appear to be those of a normal female. Nevertheless, a few patients with some masculinization of the external genitals have also ultimately become feminized at puberty. In general, however, pubertal masculinization is to be expected if there is any ambiguity of the genitals. Therefore, before puberty the estimation of future hormonal dominance may be determined by morphological studies of the genitals. If virilization is expected, castration is essential prior to puberty if the patient is to be reared as a girl, and this was routinely done in all patients of this group at the first operation.

For surgical purposes, the genitals (internal and external) of virilizing male hermaphrodites were divided into three groups as the surgical problem in each group was slightly different.

The first group (A) had tubes, uterus, and vagina, in addition to external genitals which were masculinized to varying degrees. Six of the 23 patients fell into this group.

The second group (B) had only a vagina of varying depth, usually rather shallow, in addition to external genitals which were masculinized to varying degrees. Eleven out of 23 patients fell into this group.

The third group (C) had neither tubes, uterus, nor vagina, but had external genitals which were so unsuitable for male construction that female reconstruction seemed the lesser of two difficult surgical problems. Six of the 23 patients fell into this group.

In Group A the surgical problems and potentialities with respect to the external genitals were precisely as with the virilizing female hermaphrodites. Therefore, in this group of patients all necessary surgical procedures were completed at the first operation. Needless to say, this is the most satisfactory group of male hermaphrodites for feminine orientation, as suitable estrogen therapy will

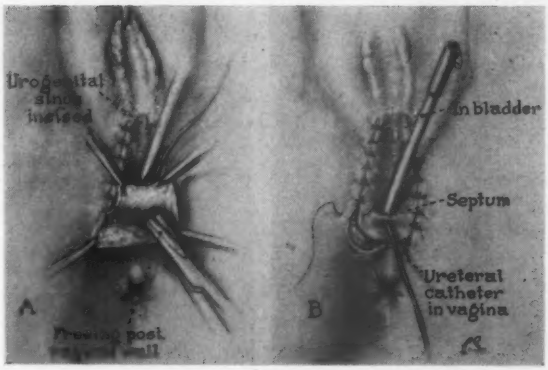


**Fig. 2.** *A*, With the catheter in place the posterior vaginal wall is freed. *B*, Completion of the operative procedure.

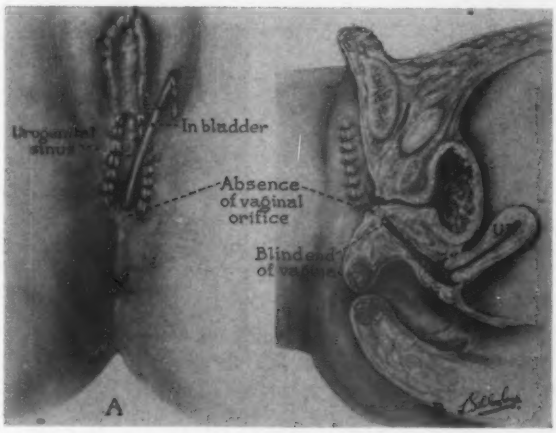


**Fig. 3.** *A*, Substantial incision in the urogenital sinus fails to reveal the vaginal orifice. *B*, Sagittal view of patient who had a noncommunication of the vagina with the urogenital sinus.

result not only in female secondary sex characteristics, but in periodic uterine bleeding as well. One of the 6 patients in this group had complete masculinization of the external genitals and was raised as a boy. The surgical procedure consisted of hysterectomy and bilateral salpingectomy and biopsy of the gonads (Figs. 6, 7, and 8).

In Group B the potentialities with respect to the external genitals were not unlike those in Group A. The special surgical problem of this group was the shallowness of the vagina in 6 of the 11 patients with the result that complete surgical treatment was impossible in infancy for these 6 patients. After full growth is attained, surgical length-

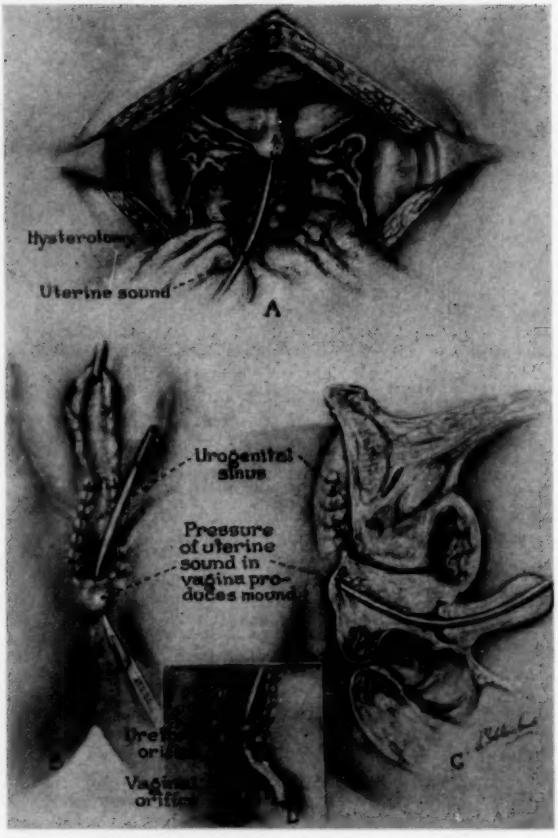


**Fig. 4.** *A,* Laparotomy in order to introduce a uterine sound through the fundus of the uterus. *B,* Tenting of the perineum by the tip of the uterine sound. *C,* Sagittal view of the same situation. *D,* Completion of operation.

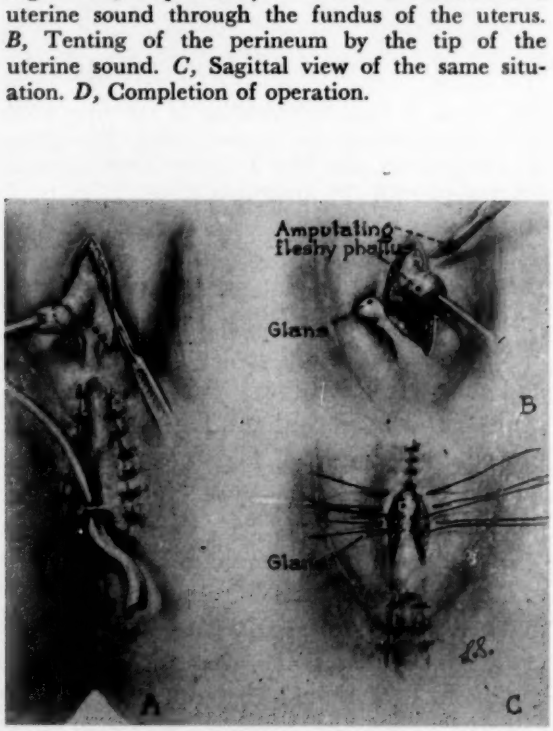


**Fig. 5.** *A, B,* and *C,* The various steps in the reduction of the size of the clitoris without removing the glans or the corpora cavernosa.

ening of the vagina, by split-thickness grafts, will be required exactly as in normal genetic females who have congenital absence of the vagina. This has already been carried out in one adult patient of this group. One patient in Group B was reared as a male. As the scrotolabial folds were often well developed in this group, considerable ingenuity was required to fashion new labia (Figs. 9, 10, and 11).

Group C represented the greatest surgical problem, as it was not always possible to achieve a completely satisfactory feminine appearance of the external genitals in infancy and there was no possibility of completing the surgical treatment until an artificial vagina was constructed after full growth was attained. In some instances the genitals were quite skimpy (Fig. 12) while in others excess tissue had to be removed.

### Male hermaphroditism with feminization

The surgical treatment of the testicular feminization syndrome was the simplest of all the major subdivisions of intersexuality (Table V). As is generally true in this syndrome, no patient had any development of the Müllerian ducts and the external genitals were entirely feminine in appearance. In only one patient in this series were the external genitals abnormal enough to require therapy. This one patient had an enlarged phallus which was excised. In 2 of the 6 patients the vagina was lengthened since it was too short to be functional.

The debatable procedure in testicular feminization is whether or not to remove the testes and, if so, when. Because of the predilection of these abnormal testes for malignant degeneration, the testes of all patients in this series were removed when the patient was first seen if she was over the age of 20 years or by that time if she was seen earlier. There is apparently only one reported case[6] with malignant change before 20 years of age, so that gonadectomy by age 20 seems a reasonable risk while retaining the testicular secretions during the important pubertal years.

### Other forms of intersexuality

The number of patients in this series with the triple X syndrome, true hermaphroditism, and gonadal dysgenesis, are too few to require separate consideration. In these groups the general principles of treatment for all hermaphrodites were applied. This implies removal of contradictory sex structures and reconstruction of the genitals along feminine lines. No problems were encountered with these patients that have not been discussed under the most common forms of intersexuality.

### Implications concerning the endocrine theory of sexual differentiation

The experience with this group of patients afforded a unique opportunity to observe the development of the generative tract in the human under a variety of abnormal conditions and to judge the findings in the light of the experimental abnormalities of laboratory animals. Space does not permit even a brief summary of the mass of data bearing on the endocrine theory of sexual differentiation, but this information is readily available in recent extensive reviews by Jost and others.[7-9] Our present purpose will be satisfied by three statements of important findings of experimental embryology as reviewed by Jost to serve as points of comparison with the findings of the human. Supporting data are available in the review by Jost.

The statements to follow are better understood if it is recalled that experimental alteration of the animals took place during the indifferent or bisexual phase of sexual differentiation. That is, when the embryo was possessed of both Wolffian and Müllerian ducts in an undeveloped state and external genitals which were capable of development in either direction.

1. In genetic males or females, in a variety of mammals and marsupials, gonadectomy prior to a critical time invariably resulted in atrophy of the Wolffian ducts, development of the Müllerian ducts, and feminine development of the external genitals. This experimental finding that female development is the asexual norm, so to speak, regardless of

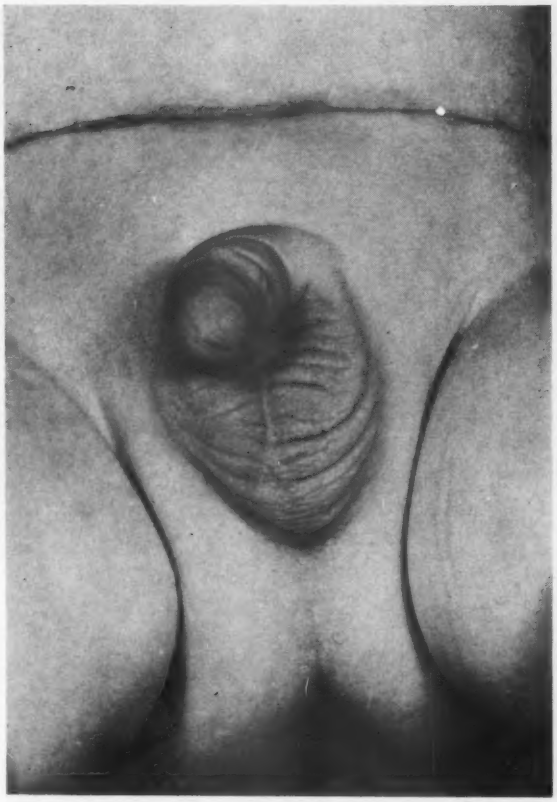

**Fig. 6.** Completely masculinized external genitals of a male hermaphrodite who was suspected of being abnormal because no gonads were palpable either in the scrotum or in the inguinal areas.

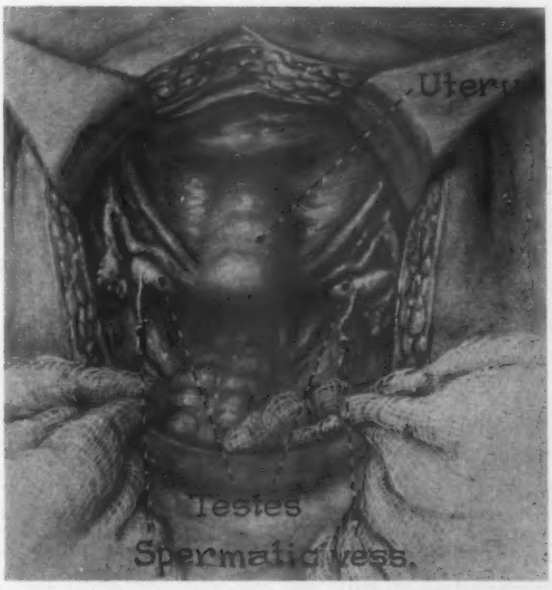


**Fig. 7.** View at laparotomy of the same patient shown in Fig. 6. The uterus and tubes are quite well developed in spite of the fact that the gonads are testes.

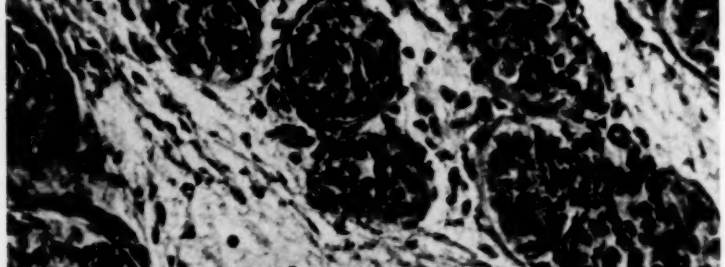

Fig. 8. Section of right testis from patient shown in Figs. 6 and 7. (Hematoxylin and eosin ×400; reduced ⅓.)

genetics, is amply paralleled in the human by the findings in patients with gonadal aplasia.

2. In genetic female mammals and marsupials, the administration of steroidal androgens to the mother will masculinize the external genitals of the fetus, will result in the persistence of some Wolffian structures, but will not inhibit the development of the Müllerian ducts into tubes and uterus. This experimental finding may now be extended to the human by a study of patients with congenital adrenal hyperplasia and with virilization due to maternal androgens. In no patient with these syndromes, in this series or in the literature, so far as we are aware, have there been any defects in the development of the Müllerian ducts in spite of partial or complete masculinization of the external genitals.

3. In genetic males experimental testicular impairment results in a spectrum of abnormalities, the most severe of which is represented by Paragraph 1 above. Castration at intervals during embryonic life shows that there is a critical stage for each part of the generative tract, by which time each structure is marked for life as masculine. Unilateral castration before the appropriate critical time will yield lateral asymmetry of the duct system but partial or complete masculinization of the external genitals. Reduction of testicular activity by hypophysectomy results in the development of Wolffian structures near the testis, Müllerian retrogression, and hypospadic development of the external genitals. These data are complicated and do not lend themselves to simple interpretation, but they do demonstrate that a normal testis is the principal evocator of normal male development and acts as a

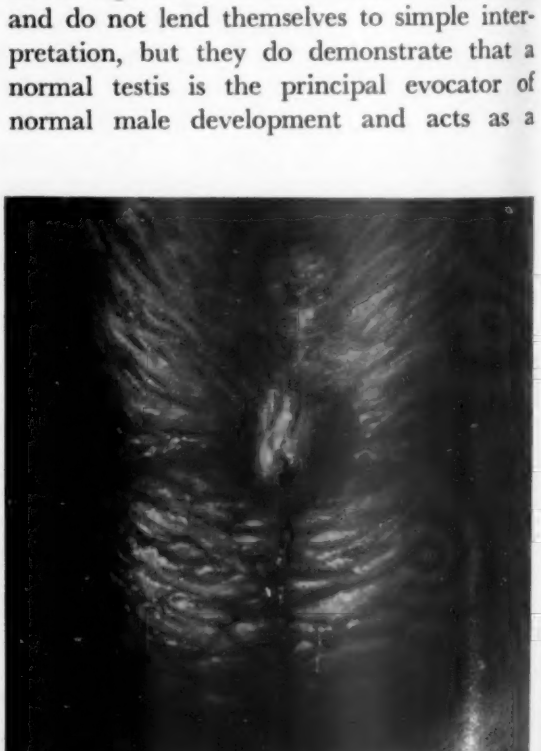

Fig. 9. External genitals of a male hermaphrodite of type B.

stimulator for the development of the Wolffian ducts and for the masculinization of the external genitals and as an inhibitor to the Müllerian ducts. In general, in the experimental animals, these actions are integrated. Thus, in the rabbit and opossum if the testis is adequate to suppress the development of the Müllerian ducts, it is also adequate to produce masculinization, at least in part, of the external genitals.

The concept that female development is the asexual norm and that the testis is the sole evocator of normal male sexual development and the further concept that various degrees of testicular impairment result in only partial masculine development can be verified in many male hermaphrodites. There are, however, two groups of human patients which represent exceptions to this general scheme of things and have no counterpart in presently available experimental data.

The first exception is represented by the testicular feminization syndrome, where, except in rare instances, during embryonic life, there is apparently insufficient androgenic secretions from the testis to cause even the slightest masculinization of the external genitals and yet, without exception, there was sufficient inhibition of Müllerian development to cause the absence of uterus and tubes in all cases.

The second exception is essentially the reverse of the first and is represented by Group A of the virilizing male hermaphrodites, where there is substantial masculinization of the external genitals, but where, in spite of this there is no inhibition of the Müllerian ducts, so that they are represented by well-differentiated tubes and uterus (Figs. 6, 7, and 8).

To explain these two exceptional human observations in terms of testicular evocation, it is necessary to postulate at least two hormonal secretions from the embryonic testis. This hypothesis presupposes one hormone concerned with masculine stimulatory development of the Wolffian ducts and external genitals and the other concerned with suppression of the Müllerian ducts. In the

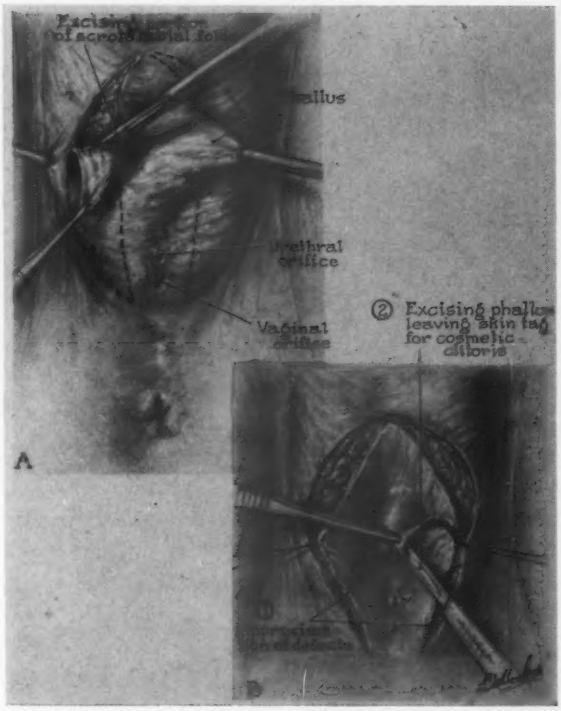


**Fig. 10.** *A,* Operative procedure to reduce the size of the scrotolabial folds and make them more feminine in appearance; the same patient as shown in Fig. 9. *B,* One and two, further steps in the operative procedure.

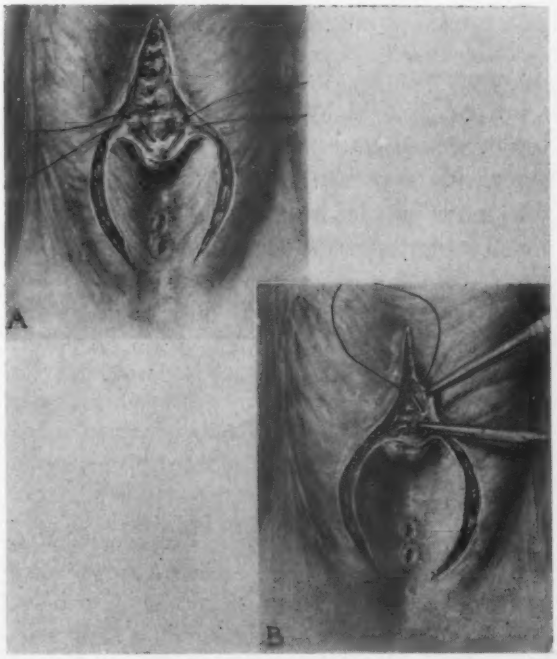


**Fig. 11.** Completion of operation removing considerable bulk from scrotolabial folds and reducing the size of the phallus without removing the glans or corpora cavernosa. In this case the vagina was 2 cm. in depth.

**Fig. 12.** The inadequate genitals of a Group C virilizing male hermaphrodite.

human material, the testicular feminization syndrome would thus result from a testis totally inadequate in the vast majority of cases with respect to the stimulatory secretion, but quite normal with respect to the Müllerian inhibitory secretion. On the other hand, the Group A virilized male hermaphrodites would result from a testis only partially inadequate with respect to the stimulatory secretion, but totally inadequate with respect to the Müllerian inhibition. Other male hermaphrodites, such as those represented by Groups B and C of the virilizing male hermaphrodites, may be partially inadequate with respect to both the stimulatory and the inhibitory embryonic secretion. The dichotomy of response between the Müllerian ducts and the external genitals is further supported by the observation alluded to above that no known steroid will inhibit Müllerian development in animal or man, but these same steroids are markedly androgenic with respect to the external genitals. This leads to further postulation that in the embryo the androgenic evocator of the testis may be steroidal in nature while the Müllerian inhibitor is probably not.

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## Discussion

Dr. H. Close Hesseltine, Chicago, Illinois. This presentation focuses attention upon some of the problems of intersexuality. These patients are catalogued in part as genetic male or female. Emphasis is made that, in the great majority of these patients, if reconstruction is to be practical, it must be toward the female characteristic. For instance, reference is made that, out of 240 consecutive cases, only 20 patients had external genitals reliably suitable for male reconstruction.

The main theme of their discussion points toward early and accurate diagnosis of the clinical situation so that the patient may be reared with the proper attitude and without the need for reversal of role.

Hypotheses explain in part the authors' concepts. One of their explanations is supported by the observation that the mothers of 16 patients

of the nonadrenal female hermaphroditic group received androgenic steroids during pregnancy.

One may be startled by the statement that on the basis of experience about two thirds of all male hermaphrodites should be reared as girls because of the difficulty in the reconstruction of the male apparatus. The male hermaphrodites numbered 21. The total number in this series of the authors was 94 patients. The authors speak of genetic females and genetic males, yet there is no reference to the confirmation that the gene composition of the cells would confirm this anatomical impression. It is suggested that in future investigations cell studies be made to actually determine the true genetic situation. This information would not be helpful in the current management but it might aid in the future in the prevention of these anomalous situations.

The illustrations and tables speak for themselves. One must recognize that these unfortunate individuals begin extrauterine life with a problem. The early recognition and a definitive commitment on these problems would contribute greatly to proper emotional and mental growth. These individuals, by proper management and surgical reconstruction, might be reasonably normal spouses, and otherwise live a useful and normal life (except for reproduction).

DR. WILLARD ALLEN, St. Louis, Missouri. It is always a pleasure to hear Dr. Jones talk about this fascinating subject in which I, too, am interested, and I would like to ask him two questions:

1. Is there any agreement as to the proper word to use in this condition?

2. A short time ago at the Jewish Hospital in St. Louis, we saw a child 2 years of age who was considered to be a female, although I do not know on what grounds because the only sign externally was a phallus sticking up, in essentially the normal position, but there was no prepuce and in the center there was a urethra through which the child voided. There was no uterus and nothing suggesting a gonad, and on biopsy of the region there was nothing but fibrous tissue. The genetic sex as determined by chromosome study was male. We really did not know what to do so we left the child as female with the expectation that the phallus would not amount to much and that at some future date it would be possible to construct a normal vagina. We thought this was gonadal agenesis. What would be your suggestions in such a case?

After asking these questions I would remark that in the last 2 years we have had two superb examples of true hermaphroditism. In one the ovaries were present and contained eggs, and in one testicular tubules were present and there was spermatogenesis. In this particular young man menstruation was occurring regularly but he desired to live as a man. Manifestly, this person should have been regarded as a female because of the normal vagina, normal uterus, and a urethral orifice which was more female than male. The interesting aspect of this case was that the young man had been able to conceal his sitting down to urinate throughout childhood and nobody in the family knew about this. The thing that brought him to us was his fear of being discovered when he was drafted into the Armed Forces. He had two problems: he did not like to menstruate and he wanted his bosoms reduced. So we worked out a plan for correction of some of his difficulties which amounted to hysterectomy. We did not tamper with the vagina because it seemed this would provide available tissue in case the urethra was corrected, but this did not bother him. He said he would never have any more operations and he was totally pleased and satisfied with having his bosoms reduced in size and his uterus removed. But he still had to sit down to urinate.

We have seen one adult who had lived as a girl but was a male hermaphrodite because at puberty he became typically male, and the question here was what to do with him. Anatomically it was feasible to build a good urethra in him. I had the good fortune to see this 20-year-old patient one day after he had his hair cut off and had moved into male clothes. This person made an extraordinary remark to me which would seem significant. The patient said, "This is the first time in my life I have ever felt like going into a drugstore and buying cigarettes." He was totally relieved by being told he was a male with the expectation that he would be able to live as a man—which he actually did because within 2 years he was married as a man and now within 5 years is divorced as a man.

DR. JOHN L. MCKELVEY, Minneapolis, Minnesota. I do not wish to discuss the paper but to ask a question. Dr. Jones has presented a fascinating hypothesis as to the background of the development of the genitals in these peculiar disturbances. We have been interested recently in a family with feminizing testicular syndrome.

There are 4 patients in this family already discovered with the syndrome and undoubtedly more to come. We have been concerned about the explanation of the development of the vagina in these people. They have no internal genitals except the testes.

There are two approaches to this problem and Dr. Jones has presented one, namely, the endocrine explanation. Now the question is, What is the embryologic explanation of the development of the vagina? Three of these 4 individuals are married. They have perfectly normal external genitals. The vaginas are complete and intact and functional. If we accept Meyer's concept of the development of the vagina, we think of it as originating from the Müllerian apparatus which reaches the urogenital sinus by following the Wolffian apparatus to that area. The final vagina is the product of active invasion of this by the urogenital sinus with ultimate formation of the vagina by its epithelium. The disturbances which are associated with abnormalities of the development of the urogenital sinus leave deficient vaginas on the basis of the concept that ultimately the vagina is derived from the urogenital sinus.

I have been embarrassed by being asked how the vaginas are formed in these people. It seems that we must either accept the fact that this is not explainable within Meyer's concept or else we must look for some other developmental feature which is quite different and distinct from this. I would be interested in having Dr. Jones discuss this or at least tell us what he knows about it or what suggestions he has as to how this comes about.

Dr. Jones (Closing). The patient described by Dr. Allen brings up an exceedingly interesting and important aspect of the theory of differentiation. You will remember that the patient was agenetic with respect to the gonads but had a phallus. The significance of this revolves around the entire concept of the theory of sexual differentiation, because by this theory one assumes that the chromosomes are important only in designating the sex of the gonad and that having designated the sex of the gonad, the development of everything else is dependent upon the gonad and the chromosomes have nothing to do with that development. Therefore, if male genitals develop in patients without gonads, this throws some doubt on the concept. That is why this patient is important. We have had 2 patients in whom we thought this was the case, but by serial section of all the material removed on both sides, we found a nodule of cells in each side not larger than 15 cells across and which was apparently composed of Leydig cells. It is also possible to hypothesize that the testes were functional in early embryonic life and subsequently degenerated for unknown reasons.

The theory of differentiation of the gonads is still uncertain, and this is brought out by the case of true hermaphroditism referred to by Dr. Allen. Most of them have a positive chromocenter. Some are said to have negative chromocenters but there is suggestive evidence to indicate that many if not all of these are chromosomal mosaics with a low chromocenter count. However, those with positive centers that have been studied have 46 chromosomes with XX configuration of the sex chromosomes. This means that in this instance alone there has been development of testes in spite of the genetic stamp of XX chromosome material.

We have a true hermaphrodite to operate upon next week and we hope to obtain a culture of the gonads, because it is possible that there is a mosaic with respect to the local development of the testes. If this is not so and if there is testicular development with XX chromosome material, we will have to look to a new concept to explain testicular development in the presence of the genetic stamp of ovarian development.

Dr. McKelvey has asked about the embryology of the vagina. We could spend the rest of the day discussing that. I will only say that it seems that by classic embryology it is difficult to understand the clinical situation he describes. It may be that we have an opportunity to use human material to interpret embryology rather than the other way around. If we approach it from this point of view, it may be that these cases are illustrating for us the development of the Wolffian portion of the vagina and that Koff was right when he said the vagina was about one third developed from the Müllerian duct from above and two thirds by budding of the urogenital sinus from below, and that these patients without development of the Müllerian duct but with vaginas do show us that the Wolffian system is entirely capable of providing at least some vaginas. It should be mentioned that a recent review of the data by Fluhmann is against this interpretation.

Dr. Hesseltine referred to the psychosexual development of hermaphrodites. He quite prop-

erly pointed out that most of them are young and we do not know the long range psychosexual development. I can only say that Dr. Money, who works with us, has carefully followed these patients and he is convinced that their reactions are in every way female, if they are reared and surgically oriented as females. This simply means, I think, that our psychosexual orientation has much more to do with the attitudes of living than it has to do with the anatomical configuration of the gonads.

Dr. Hesseltine has emphasized the importance of chromosomal studies. I can only say that we are studying these in all current patients, but do not have the data in the older patients of this series. This information will be reported later.

# The cytogenetic analysis of some disorders of sex development

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*Harwell, England*

THE subject matter of my lecture today is very largely a development of the last two years. The broad outlines of its development would appear to have been followed by medical practitioner and biologist alike. However, if we are realistic we must acknowledge that the spate of studies on the chromosomes of human subjects exhibiting congenital defects of one kind or another has provided very little help to the practicing doctor as yet: the advances have all been at a more fundamental level. So, inevitably, both for this reason and because of my training, I must approach my subject in an academic and theoretical manner.

We are all familiar with the ideas of simple Mendelian genetics, but the practice of thinking in terms of the full set of genes —the genotype—and of the changes of gene balance brought about by adding or subtracting whole chromosomes or chromosome segments may be new to some. My compatriot, C. D. Darlington, has pointed to these differences of approach by referring to the one—the Mendelian—as *analytical* genetics and the other as *integral* genetics. The integral approach is, I think, essential if we are to understand the consequences of gross chromosomal changes, among them the disturbances of the sex chromosomes that I shall discuss.

*Medical Research Council*
*Radiobiological Research Unit.*

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The concept of gene balance is usually ascribed to Bridges but one can trace the idea back to the studies of Goldschmidt on sex determination, particularly using the moth, *Lymantria dispar*. This species exists as a complex of many geographic races within each of which the sex phenotypes are clear-cut and unequivocal. But when he crossed moths from different races Goldschmidt obtained large numbers of intersexual individuals in which not only was the primary sexual anatomy ambiguous but the secondary sexual features were in varying degrees intermediate as well. Goldschmidt carried out a very extensive study of these crosses and arrived at three important conclusions regarding the genetics of sex determination. These are: (1) that the egg naturally destined to develop into an individual of the one sex nevertheless contains within it the potentiality of developing into an individual of the other sex; (2) that the phenotypic sex is determined by the quantitative relationship between the aggregate strengths of multiple male and female genetic determiners; and (3) that when these aggregate strengths are nearly equal, development may proceed as for the one sex up to a certain stage, or *turning point*, and continue thereafter as for the other.

Bridges' classical work on *Drosophila melanogaster* came a little later. He was able to show that feminizing determiners (or genes) were located in the X chromosome, masculinizing determiners in the autosomes, and that the Y chromosome had no effect on the course of sex development, though its presence was essential for the completion

of the maturation of the sperm. These conclusions followed the discovery and study of flies with abnormal combinations of sex chromosomes, work that later prompted the suggestion that certain of the abnormalities of sex development in our own species might also be ascribable to abnormality of sex chromosome constitution. This is no longer a possibility—we know it to be true, though it has taken nearly 40 years to establish its truth. The reason for this long delay lies in the fact that the technical problems involved in obtaining satisfactory preparations of human chromosomes have been resolved only within the last few years.

Before the new methods for chromosome study were introduced, however, a great stimulus to the study of errors of sex development was provided by the discovery of the sex chromatin by Barr and Bertram. The sex chromatin, as is well known, is a small Feulgen-positive body or chromocenter lying closely appressed to the nuclear membrane in a high proportion of the cells of normal females. The nuclei of normal males lack it, or, if it is present, it occurs in such a small fraction of the cells that good preparations from different sexes are always clearly distinguishable when examined by experienced workers. At one time the possibility was considered that the body might represent the two X chromosomes of the female fused together. Later evidence, however, indicates that it is more likely to be derived from only one of the X chromosomes. This chromosome, it is believed, is differentiated in its mitotic behavior from the other.

Although sex chromatin is detectable in many (not all) tissues, the first investigations were made with sections made from skin biopsy specimens. Later it was found that suitably stained smears from the buccal mucosa were equally reliable and, being simple and rapid, this became the method of choice. The study of sex chromatin brought its first important results when preparations from patients with Turner's and Klinefelter's syndrome were examined.

Patients with Turner's syndrome exhibit a feminine phenotype and are characterized by dwarf stature, a variable array of anatomic abnormalities (of which a short, webbed neck and cubitus valgus are among the most frequent), primary amenorrhea and failure of secondary sex development at puberty, raised urinary gonadotropin excretion, and the replacement of the ovaries by "streaks" of connective tissue. Yet notwithstanding the unquestioned femininity some 80 per cent of the patients were found to be sex-chromatin negative like normal males. The possibility was therefore advanced that these cases were examples of reversal of sex development, that is, of origin from XY zygotes that would normally have been destined to develop into males.

At this point an appeal was made to genetic evidence. It is common knowledge that the frequency of color blindness differs considerably between males and females. This is because of its sex-linked recessive mode of inheritance. In white populations the frequencies are about 7 per cent and 0.6 per cent, respectively. When color vision in a series of patients with Turner's syndrome was studied, it was found that the proportion of patients exhibiting color blindness approached that observed in normal males. This observation could then be construed as support for the possibility that these patients (or at least many of them) had XY sex chromosomes. It was pointed out, however, that, although the evidence indicated the presence of only one X chromosome, the data were equally consistent with a defective sex chromosome constitution including only one X and no Y.

In Klinefelter's syndrome the phenotype is male but the testes are very small and the seminiferous tubules atrophic. Associated, but not invariable, features include gynecomastia, eunuchoid habitus, raised urinary gonadotropin excretion, and mental defects. Here also the sex-chromatin observations proved to be anomalous. About 80 per cent of the patients were found to be sex-chromatin-positive like normal females. Again the possibility of origin from XX zygotes and reversal of the normally destined direction of sex development was considered as the

solution. Color vision examinations subsequently revealed a discordance with the frequency of color blindness in normal males and supported the assumption of an XX constitution.

The need for direct examination of the chromosomes in a series of these cases now became overwhelmingly strong. Fortunately, a new interest in human chromosomes had been generated by the newly reopened question of the true diploid number in man, the possibilities arising out of advances in tissue culture technique and the need to examine the chromosomes in leukemic cells. This interest has now provided us with three general methods. Bone marrow cells, white cells from peripheral blood, or small biopsy specimens from skin or (if available) a deeper-seated organ or tissue are incubated or cultured in vitro for a few hours to several weeks. Once there is a sufficiency of mitoses in the suspensions or cultures, a variety of procedures can be followed. All employ devices to disperse the chromosomes widely in the cytoplasm. The final aim is to obtain preparations with the cells distributed in a single layer. In the best preparations there are at least some cells in which the chromosomes are evenly distributed and lying in a single plane, without overlaps.

Once efficient methods had been introduced, the problems of the sex-chromatin anomalies in Turner's and Klinefelter's syndromes were quickly solved. The chromatin-negative patients with Turner's syndrome were found to have 45 chromosomes, including only one presumptive X and no Y; the chromatin-positive patients with Klinefelter's syndrome had 47 chromosomes, with two X chromosomes (as had been anticipated) but including a Y chromosome as well. It is convenient to express the sex chromosome constitutions of these two abnormal types as XO and XXY, respectively.

The chromatin-negative patients with Klinefelter's syndrome who have been investigated appear to be chromosomally normal (XY) males. A difference in testicular histologic findings has been claimed for this group and they may be etiologically distinct.

One of the first cases of a chromatin-positive patient with Klinefelter's syndrome to be studied revealed a further anomaly. Most of the cells contained 47 chromosomes and had a presumptive XXY constitution, but there was a considerable minority of cells with a count of 46. Analysis of these cells showed that nearly all of them were compatible with an XX constitution. The patient thus appeared to be a mosaic of XX and XXY cells. When chromatin-positive patients with Turner's syndrome were examined, this phenomenon was again encountered. This time the mixture was of presumptive XO cells with 45 chromosomes and presumptive XX cells with 46 chromosomes.

The first mosaic individuals encountered were identified from study of the chromosomes in bone marrow cells alone and the possibility had to be considered that the observed anomaly was confined to that tissue. An origin after birth, even in adult life, could not be excluded by the evidence. However, other examples are now on record in which the same mixture of cells has been found in two or more tissues or regions of the body. These suggest origin at an early stage of embryogenesis, possibly even at the first division of the zygote. Either of two types of mitotic error might be implicated. One or both disjoining chromatids of one X chromosome might fail to reach the pole and be excluded from the daughter nuclei at telophase. Alternatively, both chromatids might proceed to the same pole so that one daughter nucleus contained an extra chromosome and the other one less than the normal number. We refer to these errors as lagging (or mitotic loss) and mitotic nondisjunction, respectively.

The discovery of mosaicism leads us to a possible explanation of the origin of the anomalous XO Turner and XXY Klinefelter constitutions. An early mitotic error in otherwise normal XX or XY embryos could give XO cells, and if these alone were included in the embryo proper, XO individuals would result. At present, the possibility cannot be excluded that many, even all, XO individuals arise in this way. One may point to the evi-

dence from the house mouse where XO females appear spontaneously. In this species it is possible to identify the parental X chromosomes by appropriate genetic markers and so to show that in the XO animals it is invariably the paternal X that is missing. Other evidence strongly suggests that the mechanism involved is mitotic loss during the first division of the zygote.

The XXY constitution could also arise through an early mitotic error. Nondisjunction of the X in a XY zygote would yield XXY and YO cells, and the YO cells, lacking even one X chromosome, we may presume to be inviable. It is possible to put forward an argument from the color blindness data to show that this is unlikely to be the only mechanism of origin of XXY individuals. Were it so, the two X chromosomes would be genetically identical since both would be derived mitotically from the one that entered the zygote. We know that about 7 per cent of X chromosomes bear a recessive gene for color blindness. It follows that 7 per cent of XXY patients with Klinefelter's syndrome should be color blind if the assumption we are testing is true. Yet, of the 107 chromatin-positive patients with Klinefelter's syndrome who are stated in the literature as having been tested only 3 were color blind. At least some, it would seem, must bear two X chromosomes of independent origin, a situation which could come about only through the contribution of the two sex chromosomes to the zygote by one gamete. Theoretically, this could be either an abnormal XX-ovum or an abnormal XY-sperm, either of which could arise as a consequence of nondisjunction during gametogenesis.

Meiotic nondisjunction is a well-established phenomenon from both cytologic and genetic evidence in lower animals and in plants. We can be sure that in our own kind and in other mammalian species the problem will be not whether it occurs or not, but how frequently it occurs. Moreover it could involve either or both sex chromosomes and happen at either or both of the meiotic divisions, so yielding a great variety of theoretically possible gametic types. The products of their union with normal gametes of the opposite type and with each other would then lead to an even greater array of expected zygotic types.

Let us consider only the two kinds of nondisjunctional gamete already mentioned, namely, XY-sperm and XX-ova, together with their complementary products, which we may express as O-sperm and O-ova, respectively. Union with normal X-ova and normal X- and Y-sperm would yield the XO and XXY Turner and Klinefelter constitutions already discussed and also three new types, YO, XXX, and XXXY.

There are good general grounds for believing that YO zygotes, even if they underwent the early cleavage divisions and implanted, would not develop very much further and would then be resorbed or aborted.

Triplo-X individuals, however, are known. They are females and apart from menstrual disturbances in some cases they are clinically unremarkable though mental performance may be impaired. The first example of this condition to be detected presented as a case of secondary amenorrhea and it was only after the chromosomes had been examined that it was discovered, independently, that there were *two* sex-chromatin bodies in many cells of the buccal mucosa. The other cases so far published have been identified in the course of sex-chromatin surveys of the inmates of mental institutions. Several of them have borne children. Now three chromosomes of the same kind would be expected to form a trivalent association during the prophase of meiosis, which would then be expected normally to disjoin two to one pole, one to the other, at first anaphase. Sometimes, however, one of the three might lag behind and be excluded from both daughter nuclei. We may therefore expect a triplo-X mother to produce both normal X-ova and abnormal XX-ova, the normals perhaps being slightly in excess. She should then have nearly equal chances of bearing normal sons and daughters and of bearing XXY Klinefelter sons and XXX daughters

like herself. However, all the 10 children of triplo-X mothers whose sex-chromatin has been examined and recorded are apparently normal for their sex. So far, then, there is no evidence that the process of secondary nondisjunction, as it is known to drosophilists, occurs in these women at all.

Formally, the cytogenetic situation in Mongoloid mothers is very similar. One would expect normal ova, and ova with an extra chromosome 21, to be produced in approximately equal numbers. The children of Mongoloid mothers should therefore include Mongols and normals in about equal numbers. My colleague, Dr. Clarke, has made an enumeration of the children of Mongoloid mothers that are recorded in the literature. She found that 11 mothers had borne 8 normal children and 5 Mongols. These figures are consistent with the expected 1:1 ratio and demonstrate that secondary nondisjunction of the extra chromosome does occur in Mongoloid females.

The sex-chromatin surveys of the inmates of mental institutions have also been principally responsible for the other types of numerical sex-chromosome unbalance that have been discovered. There are 4 of them, namely XXXX, XXYY, XXXY, and XXXXY. Two XXXX cases have been reported. They are females, and, like the triplo-X cases, do not exhibit any obvious anatomic abnormality. All the others are males and all may be regarded as exhibiting variations of Klinefelter's syndrome. The degree of mental impairment, however, is generally greater and some of them exhibit skeletal defects. It would strain credulity to suppose that *all* these cases originated through mitotic errors during early embryogenesis, though in each it is a formal possibility. The alternative is nondisjunction during gametogenesis. To account for the 48- and 49-chromosomes cases, two and three separate abnormal events have to be postulated either in gametogenesis or embryogenesis, or partly in one, partly in the other.

Perhaps the most important point to emerge from this parallel study of sex-chromatin and chromosomes is the empirical rule that the maximum number of sex-chromatin bodies is one less than the number of presumptive X chromosomes. The rule holds whether the phenotype is female or male. It accords well with the assumption that each sex-chromatin body represents a single sex chromosome.

The seven types of simple numerical disturbance of the sex chromosomes do not exhaust the irregularities that have been discovered. In addition to the XX/XXY and XO/XX mosaic types several other mosaic combinations are known including XO/XY, XO/XXX, XY/XXY, and one with three components, XO/XX/XXX.

Two other types of irregularity involve morphologically abnormal presumed derivatives of the X-chromosomes. Both were found in females. In the first case to be described the one presumptive X chromosome was replaced by a much smaller chromosome. This chromosome might have originated in several ways but the simplest assumption is that it arose by loss of the greater part of the longer arm. Several examples of the second type are known. The one X chromosome is again replaced, this time by a longer chromosome with equal arms. The most plausible explanation is that the abnormality arises by symmetrical duplication of the longer arm of an X chromosome about its centromere. The two arms would then be morphologically and genetically homologous. Such isochromosomes are known in other species. All the patients in whom they have been identified exhibit Turner's syndrome and are chromatin-positive.

The cases just discussed are too heterogeneous in their clinical features to permit any useful conclusions as yet. Nevertheless, we may hope that further study of the phenotypes associated with mosaicism and with presumed derivatives of the X chromosome will reveal more about its function and perhaps also of its component parts.

All the cases (other than one mosaic) I have mentioned so far can be encompassed within a simple generalization: all that carry

a Y chromosome exhibit a male phenotype and all that do not carry a Y chromosome exhibit a female phenotype. The XXXXY cases indicate that the Y is sufficiently active to ensure masculine development even in opposition to four X chromosomes.

The classic experiments carried out by the French embryologist, Jost, have shown that if the gonads of a developing rabbit embryo are ablated at a suitably early stage the embryo subsequently develops female internal and external genitals irrespective of its naturally destined sex. Other experiments by Jost lead to the conclusion that the development of male genitals is dependent upon the presence of testes. The transformation of the embryonic Wolffian duct into epididymis, vas deferens, and seminal vesicle appears to be the consequence of a local evocating action of the testis on the same side of the body. Differentiation of the external genitals, on the other hand, though still dependent on the presence of testes, appears to be mediated through the action of circulating substances in the blood stream.

Viewed against this background, it may be supposed that the female is the basic or neutral phenotype and that the role of the Y chromosome is to convert the primordial, indifferent gonads into testes.

Unfortunately for this hypothesis (or so it would seem at first sight), two types of female are known who have chromosomes that are indistinguishable from the normal male set. The one condition is the syndrome known as testicular feminization. The outstanding features of this syndrome include the presence of testes (commonly in inguinal hernias), male internal genitals, short blind vagina, absent uterus, feminine external genitals, and typically feminine secondary sexual development at puberty, though with absent or very sparse pubic and axillary hair. The affected individuals are sex-chromatin negative, as their chromosomal constitution would suggest and—a point of considerable interest—the condition is hereditary, being transmitted through carrier mothers. The pedigree data are consistent with the assumption that a single Mendelian gene is responsible for the abnormality, but do not permit discrimination between the alternatives of a sex-linked recessive gene and a sex-limited autosomal dominant gene.

These facts are readily reconcilable with the postulated role of the Y chromosome as initiator of testicular development. Testes *are* present in the affected individuals and differentiation of the Wolffian duct derivatives proceeds as would be expected; the errors lie in the failure of masculine development of the external genitals and the feminine direction of secondary sex development. The importance of the genetic evidence is that it points to a unitary biochemical lesion as the primary cause of the defect and suggests that a single mechanism is responsible for the two separate phases of abnormal development. This could be the production and release of an abnormal substance by the testis, or defective response by the target organs. At the present time there is no evidence favoring either one or the other.

The second type of XY female exhibits the syndrome of pure gonadal dysgenesis. Only few patients with this condition have been described. They are not dwarfed as they are in Turner's syndrome, nor do they exhibit any of the congenital anatomic defects associated with this condition. They may be somewhat tall and eunuchoidal, and there is failure of menstruation and of secondary sex development at puberty. Where laparotomies have been performed the gonads are found to be replaced by streaks of connective tissue. Fallopian tubes, uterus, and vagina are present. The point of most interest for our purpose is that some are chromatin-positive and others chromatin-negative. The possibility has been advanced that they represent the natural analogue in our own species of Jost's rabbits with ablated gonads—that there is primary failure of gonad formation so that the neutral feminine phenotype develops whether the individual has an XX or an XY sex chromosome constitution. This idea was earlier put forward in connection with Turner's syndrome when it was discovered that both chromatin-positive and chromatin-negative cases occurred, but had

to be abandoned when it became evident that the great majority of patients with Turner's syndrome are chromosomally abnormal. At the present time its application to pure gonadal dysgenesis is supported only by a single published case, though it is expected further evidence will appear in print shortly. If the interpretation is sound our hypothesis linking the Y chromosome with testicular differentiation is unaffected, since the Y cannot transform the primordial gonad into a testis if there is no primordial gonad to transform.

Discussion of the cases of testicular feminization and pure gonadal dysgenesis therefore still leaves us in a position to assert that presence of a Y chromosome is necessary for testis formation and masculine development, even though it does not ensure it. The occurrence of true hermaphroditism, however, presents greater difficulties for the hypothesis.

True hermaphroditism is defined as the presence of both ovarian and testicular tissue in a single individual. There may be ovary one side and testis the other; either, and an ovotestis; or two ovotestes. Moreover, the cases present a bewildering variation of genital anatomy. From the genetic point of view, two mutually exclusive hypotheses are possible. Either the gonads (or gonad regions) differ because the cells of which they are composed are genetically different, or they differ notwithstanding the genetic identity of their constituent cells.

The first is a hypothesis of mosaicism. Gross chromosomal mosaicism should be detectable by present methods; finer differences of chromosome structure and differences of genetic constitutions would escape us. As with several of the other conditions we have discussed, both chromatin-positive and chromatin-negative cases of true hermaphroditism exist. Most, however, are chromatin-positive and the first 6 cases in which the chromosomes were studied were of this type. All were reported to have 46 chromosomes, including two presumptive X chromosomes, as in normal females. However, in each case only a single tissue (skin, blood, or bone marrow) was used as the source of material. Although it may not be very likely, the possibility that they were, in fact, XX/XXY mosaics, is not excluded. In such a mosaic it is reasonable to suppose that XX cells could produce ovarian tissue and XXY cells testicular tissue. That gross chromosomal mosaicism must be taken seriously as a possible explanation, at least in some cases, became evident when the first chromatin-negative case was studied and proved to be an XO/XY mosaic. Much more information will be needed before we can really assess the significance of mosaicism in relation to true hermaphroditism. Since many of the cases are subjected to exploratory laparotomy, opportunities will occur to set up cultures for chromosomal study from the abdominal organs including, most important of all, the gonads themselves. They should not be lost.

The alternative to some form of mosaicism, genetic or chromosomal, is to go back to Goldschmidt's conclusions and assume that both masculinizing and feminizing factors of varying total strengths are present in the chromosomes of both male and female, but that normally the one set decisively out-balances the other. Should, however, the balance be equivocal, it is conceivable that chance local factors operating during embryogenesis could tip the scales in different directions in the two gonads, or even in different parts of the same gonad. Ovary and testis, or separate gonadal regions of ovarian and testicular tissue, would then arise within the same individual from cells of the same genotype.

If this interpretation of true hermaphroditism is correct even for some of the XX cases, the simple hypothesis according to which the Y is responsible for testicular development would have to be rejected. The idea, however, could be retained in a somewhat more sophisticated form by assuming that the Y, though not their only seat, bears potent masculinizing factors, normally sufficient to overbalance the sum of all the feminizing factors by which they are opposed. The very rare exceptions would then express

themselves as chromatin-negative cases of true hermaphroditism. By the same token, feminizing factors would normally overbalance masculinizing factors in XX individuals, the exceptions developing as chromatin-positive true hermaphrodites.

If primary sex determination is quantitative, as this interpretation requires, it is possible to conceive of departures from the normal levels great enough to result in an excess of factors contrary to expectation from the sex chromosome constitution. Functional females might then develop from XY zygotes and functional males from XX zygotes. I hasten to add that there is as yet no evidence that such cases exist.

In 2 years much has been accomplished by the application of cytogenetic methods to the problems raised by abnormal sex development in man. These findings I have tried to fit together into an intelligible scheme, in places, some may have felt, molding them more closely to hypothesis than the facts warrant. However, a valuable function of hypotheses is to spotlight facts that do not easily fall into place and so to focus attention on points requiring further investigation. True hermaphroditism is an example. Much can still be done by thorough cytogenetic study of appropriate cases but it may well be that other and more subtle methods will be required before the problem is resolved.

I feel that I should end on this note of uncertainty, since it represents the essential feature of scientific advance. As soon as new techniques are developed and some old problems solved, new problems, or new aspects of old problems, emerge to maintain the challenge to our wit and understanding.

# OBSTETRICS

## Tubal pregnancy following treated genital tuberculosis

### Report of 2 cases and review of literature

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IT IS now generally recognized that genital tuberculosis is not a cause of absolute sterility, although pregnancy is rather uncommon in these patients.

The advent of specific antibiotics and chemotherapeutic agents has undoubtedly had a favorable effect in genital tuberculosis and, since this form of treatment has been well established, the number of pregnancies reported in patients with a past history of tuberculosis is increasing.

Shapiro,[11] in a review of the literature from 1952 to 1957, found 38 cases of pregnancy following treated genital tuberculosis and added 1 of his own. There were 12 intrauterine term pregnancies, 11 spontaneous abortions, and 16 tubal pregnancies. Since then, 2 further cases have been reported by Dexeus and Dexeus,[3] both tubal pregnancies. O'Driscoll[9] reported 1 case and quoted 2, all tubal pregnancies. Dalsace and Weibel[2] reported 6 cases of ectopic pregnancy out of 42 cases of treated latent genital tuberculosis. Patat[10] reported 1 case of full-term pregnancy after treated genital tuberculosis.

With the addition of the 2 following cases of tubal pregnancy, the number of cases of pregnancy following treated tuberculosis that I have found is 13 intrauterine term pregnancies, 11 spontaneous abortions, and 29 tubal pregnancies.

There is an undoubted predominance of ectopic pregnancies. It is the intention of this report to emphasize the role of treated genital tuberculosis as an etiological factor in tubal pregnancy.

**Case 1.** Mrs. R., aged 30, was first admitted on June 25, 1955, complaining of severe dysmenorrhea and sterility for 8 years. She gave a history of regular menstruation from the age of 17 but had had a myomectomy performed in 1952 for a uterine fibroid.

A recent chest x-ray examination showed no evidence of past or present pulmonary disease.

Vaginal examination revealed a small anteverted mobile uterus with thickened tubes and cystic ovaries. A preliminary dilatation and curettage was carried out prior to a laparotomy which showed both tubes to be distended, thickened, and nodular. The appearances were most

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suggestive of a tuberculous infection, and a small portion of the fimbrial end of the left tube was removed for histological examination.

Histological examination confirmed the diagnosis of tuberculous salpingitis, and guinea pig inoculation with the curettings gave a positive result.

The patient was discharged on July 10, 1955, and was given streptomycin, 1 Gm., and isonicotinic acid hydrazide, 150 mg. daily, for 3 months.

On Oct. 1, 1955, vaginal examination showed the uterus to be anteverted and mobile with only slight thickening of the appendages. The patient had no complaints at this time.

A further dilatation and curettage was carried out on December 15, but the curettings did not show any evidence of tuberculosis either on histological examination or on guinea pig inoculation.

The patient was seen on several occasions over the following 2 years and on Sept. 10, 1956, and Nov. 11, 1957, further curettage showed no evidence of tuberculosis either on histological examination or guinea pig inoculation.

At a routine visit on June 20, 1958, the patient complained of slight irregular vaginal bleeding from May 23, 1958. The last normal menstrual period commenced on May 3, 1958. On vaginal examination a swelling the size of an orange was found in the right appendage. For domestic reasons the patient postponed her admission until July 15, 1958, when a laparotomy confirmed an ectopic pregnancy in the right tube. The diagnosis was confirmed by histological examination of the removed appendage, which did not show evidence of tuberculosis. The patient made an uncomplicated recovery.

**Case 2.** Mrs. D., aged 29, had an appendectomy in September, 1951. At operation a diagnosis of tuberculous peritonitis was made and histologically confirmed by mesenteric biopsy.

A chest x-ray examination at this time showed tuberculous infiltration of the left apex and she subsequently received sanatorium treatment for the following 6 months. She received advice not to become pregnant for 3 years.

On June 12, 1956, the patient first attended the Gynaecological Clinic complaining of an aching pain in the right side of the abdomen and sterility. The menses were regular and she had been attempting to conceive over the past year. On vaginal examination no abnormality was found except for a slight thickening of the right appendage. On Aug. 14, 1956, a hysterosalpingogram appeared to show bilateral tubal obstruction. Dilatation and curettage revealed a rather scanty endometrium. Histological examination and guinea pig inoculation confirmed the spread of the tuberculous process to the genital tract. A further 4 month period of treatment in a sanatorium was instituted during which time she received streptomycin, INAH, and PAS. Upon completion of the treatment further curettage was performed but no evidence of tuberculosis was found on either histological examination or guinea pig inoculation.

On March 18, 1959, the patient was admitted to the hospital with a history of lower abdominal pain and 7 weeks' amenorrhea. The vaginal findings were not conclusive and the differential diagnosis lay between an ectopic pregnancy and a recurrence of the tuberculous peritonitis. A course of streptomycin was commenced but, because the abdominal pain persisted, a laparotomy was performed on March 26, and a left tubal pregnancy was removed. Her recovery from the operation was satisfactory. There was no histological evidence of tuberculosis in the removed tube. It is of interest to note that the tubal pregnancy was on the opposite side to the ovary containing the corpus luteum of pregnancy.

## Comment

Prior to the treatment of pelvic tuberculosis with modern specific therapy, the occurrence of ectopic pregnancy and tuberculous salpingitis was considered to be a very rare condition. Stevenson and Wharton[12] stated that they believed it to be one of the rarest combinations seen in gynecology. They reported only 1 case out of 402 cases of tuberculous salpingitis and 516 cases of tubal pregnancy.

Burns and Burns[1] reported 2 cases of ectopic pregnancy associated with tuberculous salpingitis in 26 cases of tuberculous salpingitis and 300 cases of ectopic pregnancy, and Stoddard[13] found 1 case in 17 cases of tuberculous salpingitis and 179 cases of ectopic pregnancy.

Kistner, Hertig, and Rock[6] report 1 case of ectopic pregnancy in association with tuberculous salpingitis out of a total of 197

cases of tuberculous salpingitis and 313 cases of ectopic pregnancy. These authors extensively reviewed the literature and found only 41 such cases previously reported and with their own case made a total of 42 cases up to 1951.

At the present time the combination of tuberculous salpingitis and tubal pregnancy should be considered even more rare as a result of healed tuberculosis by specific therapy.

From these figures it is obvious that the incidence of ectopic pregnancy in association with untreated tuberculous salpingitis is low. It is unlikely that the patients in any of the cases reported up to 1951 would have received specific antituberculosis therapy as the diagnosis was supposedly made only after histological examination of the removed tube.

That the association of untreated tuberculous salpingitis with ectopic pregnancy is rare can be explained by the fact that there is a high incidence of tubal blockage, the diseased endosalpinx is unsuited to implantation of the fertilized ovum, and there is often absence of ovulation in patients with tuberculosis.

However, following treatment of tuberculous salpingitis with specific therapy a different state of affairs obtains.

As early as 1952 Krohn, Priver, and Gotlib[7] found that the whole incidence of tubal pregnancy was increasing as a result of penicillin therapy. The incidence in private practice at that time was three times as great as in patients attended in hospitals and almost four times greater than in private practice 10 years prior to the use of penicillin. They postulated that tubal occlusion may be prevented or patency restored as a result of the specific therapy. They also quoted Eastman as saying that penicillin when administered after the development of irreversible structural changes in the endosalpinx might increase the incidence of ectopic gestation.

The structural changes in the majority of nontuberculous infections of the tubes is mostly interstitial in type but in tuberculous infection the main and primary lesions are in the mucosa, though in all infections there is ultimately a more or less severe involvement of the mucosa, muscularis, and serosa. Thus, the effects of specific therapy must be similar in tuberculous and nontuberculosis infection of the Fallopian tubes.

A considerable number of cases of genital tuberculosis are nowadays diagnosed during a routine investigation of patients complaining of sterility. Apart from the complaint of sterility, the patient has no other symptoms and both clinical and pelvic examinations are usually negative. It is therefore likely in such cases that the tuberculous process is in its early stages or in a quiescent phase, but although specific therapy is commenced reasonably early it is not sufficient to restore the normal architecture, and irreversible damage will persist after healing takes place.

Halbrecht,[4] the first who stressed the role of anti-tuberculous therapy in tubal gestation, thinks that treatment produces results similar to spontaneous healing, which he believes to occur more often than is generally realized.

As we are aware, the structural changes in the tubes following healing are seen in the formation of blind passages due to adhesions between the mucosal folds, the loss of ciliated activity, and in the residual perisalpingitis with alteration or loss of peristaltic function. These changes will prevent or impede the passage of the fertilized ovum through the tube, and with increasing trophoblastic power of penetration due to the delay, the necessary conditions as outlined by Novak[8] for the establishment of a tubal pregnancy are fulfilled.

That antituberculosis therapy is an etiological factor in tubal gestation can be seen from the number of cases now being reported. In the series presented by Halbrecht[4] he found 16 tubal pregnancies in 14 patients out of 100 women who had received treatment for pelvic tuberculosis. In the same series a further 6 patients conceived, 3 had miscarriages, and 3 had full term intrauterine pregnancies. A number of cases of repeated tubal gestations following specific

therapy have also been reported which tends to lend further support to the view that treatment is probably an etiological factor. Two such cases were described in Halbrecht's series,[4] and Haines[5] quotes 1 patient who had 3 tubal pregnancies during the course of treatment. Dexeus and Dexeus[3] reported a case of 2 tubal pregnancies, but 1 of the pregnancies occurred before antituberculosis therapy was given.

As a result of his experience, Halbrecht[4] is of the opinion that any pregnancy occurring after specific therapy for genital tuberculosis has, a priori, a 4 to 1 chance of becoming a tubal pregnancy or ending in miscarriage. In view of these facts, it would appear that there is a place for bilateral salpingectomy when the diagnosis of pelvic tuberculosis is first made at operation or following laparotomy for a tubal pregnancy in a case of treated pelvic tuberculosis. It should not require much hesitation in doing so in a parous patient or in cases where the genital tuberculosis has involved the endometrium. It seems that when the endometrium is involved, the possibility of successful pregnancy is very poor; in the majority of reported cases of full-term intrauterine pregnancies following treated genital tuberculosis the previous anatomical lesion of the endometrium had not been proved, the diagnosis being made by culture of menstrual discharges or other bacteriological means. When the endometrium is involved, tubal pregnancy or miscarriage appears, by far, to be the most common outcome.

## Summary and conclusions

Two cases of tubal pregnancy following therapy for genital tuberculosis are reported and the pathogenesis of the condition discussed.

In doubtful cases of ectopic pregnancy, a history of treated pelvic tuberculosis should make the diagnosis more certain.

The performance of bilateral salpingectomy in the treatment of genital tuberculosis in certain cases is considered.

In spite of the fact that tubal pregnancy is considered a "complication" of specific therapy of genital tuberculosis, treatment in these cases is certainly indicated.

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# Histologic study of uterine tubes with tubal pregnancy

## A search for evidence of previous infection

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THE exact etiology of tubal pregnancies is unknown; numerous theories have been proposed to explain its occurrence. The more commonly suggested factors which could delay or hinder the passage of the fertilized ovum into the uterine cavity include chronic inflammatory disease of the tubes or residues thereof, congenital anomalies such as tubal diverticula, external migration of the ovum, and extrinsic or intrinsic tumors involving the tube. In addition, endometriosis of the tube has been suggested as an etiological factor. Other etiological ideas that have appeared in the English literature in the past 60 years are a hereditary or familial tendency,[9] an impairment of function of the ciliated epithelium on the basis of absence of nerve impulses due to a "depressor neurosis,"[30] the use of oxytocic drugs,[31] profound defects in metabolism (nutritional basis),[2] and deficient tubal peristalsis (hormonal basis).[38]

The most widely accepted theory for the etiology of tubal pregnancy is that of residues of tubal inflammatory disease, usually gonorrheal, but including puerperal, postabortive, and postoperative infections. These infections play their etiological roles in probably one of two ways—either by the production of mechanical obstructive "pockets" or impairment of propulsive forces of the tube. The ovum is believed to be transported along the tube by both ciliary and tubal peristalsis. Consequently, if the ciliated epithelium and/or tubal musculature were completely or partially destroyed or innervation lost secondary to infection, it has been postulated that as a result a fertilized ovum would become imbedded in tubal mucosa.[3] To verify this postulate, physiologic studies determining the propulsive forces of the tube would have to be done, and we know of no such experiment or a practical means of so doing.

Two methods have been suggested by which chronic inflammatory disease could cause mechanical obstruction of the tube. Extrinsically, peritubal adhesions causing kinking of the tube could result in narrowing of the tubal lumen. Such a reduction in size of the lumen would be difficult to prove, both grossly and histologically, because of distortion of the tissues caused by the preg-

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nancy itself. Intrinsically, endosalpingitis with denudation of tubal epithelium may result in adhesions between plical folds of the tubal mucosa. Because of these adhesions between plica, there is a formation of glandlike spaces which result in blind pockets into which the fertilized ovum could be trapped and become implanted. It is this role of chronic inflammation, or "follicular salpingitis," which is generally considered the most important etiologically.

Many articles have appeared in the English literature in the last 55 years on tubal pregnancy, most of which deal primarily with the clinical aspects of this entity. However, some of these clinical articles include data which are presented to corroborate or dispute various etiological ideas. These data, in connection with the residues of pelvic inflammatory disease theory, fall into one of three groups: the patient's history, gross findings, or microscopic evidence of previous infection. Only three articles[12, 15, 49] were found which were devoted exclusively to microscopic study of gravid tubes.

To determine previous pelvic infections or the possibility of same, various authors have recorded the frequency of a preceding history of either pelvic inflammatory disease, abortions, or operations. A few of these series are presented in Table I.

The figures, quoted for the frequency of past pelvic inflammatory disease, should probably be the most important and here we see a great variability ranging from 0.5 per cent to 82.4 per cent. We feel that this in itself points out the obvious deficiency of depending on historical data in determining possible etiology.

The percentage of gross pathologic findings at the time of operation indicative of chronic inflammation of pelvic structures and mainly of the uterine tubes, has been noted by the following authors: MacVine and Lees,[28] 8 per cent; Urdan,[43] 10.33 per cent; James and Lafferty,[22] 12 per cent; Thorton,[41] 13.4 per cent; Lavell,[27] 19.7 per cent; Wood and Martyn,[48] 29 per cent; Varner and Green,[44] 40 per cent; Farell and Scheffey,[14] 40 per cent; Behney,[5] 42 per cent; Willis,[46] 47 per cent; Dougal,[10] 50 per cent; and Allen,[1] 50 per cent.

Although it can be inferred either from the history or from gross evidence that infection could cause changes of the tubal wall responsible for ectopic gestation, the real proof of these changes requires microscopic analysis. A total of 42 articles was found in which this was done. Of these 42 papers, 26 either did not state that the findings of salpingitis were specifically of a residual nature or were vague in some other aspect. The findings of the remaining 16 articles definitely stated that there was evidence of chronic inflammation. The percentage of gravid tubes showing chronic inflammatory changes are listed as follows: Hu,[20] 19 per cent; Thompson,[40] 20 per cent; Ingraham,[21] 25 per cent; Henderson and Bean,[18] 27 per cent; Brown,[6] 33 per cent; Tenney,[39] 33 per cent; Langman and Goldblatt,[26] 36.8 per cent; Jarcho,[23] 38.8 per cent; Cain and Walker,[7] 42 per cent; Zeliff and associates,[50] 42 per cent; Johnson,[25] 51 per cent; Wist,[47] 55 per cent; Yuan and K'uei,[49] 63.7 per cent; Falk,[12, 13] 95 per cent; and Gardner,[15] 95 per cent.

**Table I.** Per cent of series with history of pelvic inflammatory disease, abortions, and/or operations

| *Author* | *Pelvic inflammatory disease (%)* | *Abortions (%)* | *Operations (%)* |
|---|---|---|---|
| Graham[17] | 36 | | |
| Gordon[16] | 28 | 56 | 23 |
| Sellers and Sanders[34] | 28 | | |
| Masson[29] | 46.9 | | |
| Scheffey and co-workers[32] | 30.5 | | 37.7 |
| Sessums[35] | 82.4 | | |
| Echols[11] | 8 | 28 | |
| Tiemeyer[42] | 18.1 | | 53 |
| Schoregge[33] | 0.5 | 39 | |
| Johnson[24] | 22 | 18 | |
| Silberblatt[8] | 18.6 | | |
| Smith[36] | 50 | | |
| Soisson and Moran[37] | 14 | 26 | 38 |

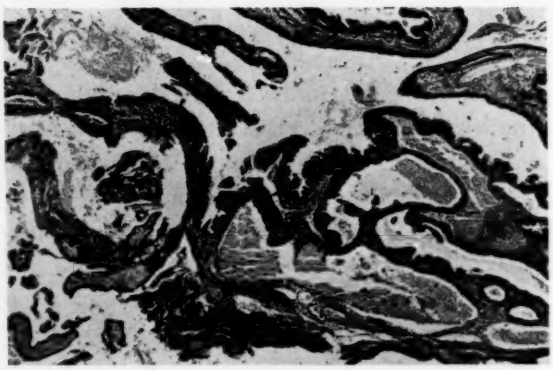


**Fig. 1.** A tubal pregnancy with placental villi and trophoblastic cells in left lower part of photograph. This tube was considered to show a mild degree of follicular salpingitis. (Original magnification, ×70.)

**Fig. 2.** A tubal pregnancy with placental villi and trophoblastic cells to the right and wall of the tube to the left showing a marked degree of follicular salpingitis. (Original magnification, ×70.)

## Materials and methods

The slides used in this study were taken from the files of the laboratory of the Department of Obstetrics and Gynecology of Northwestern University Medical School. Tissue sections were examined by each of us independently and in those few cases where there was disagreement in interpretation, slides were studied together and agreement achieved.

Slides of 121 patients were found suitable for our study. Slides from another 25 patients were excluded from our study because of the lack of adequate endosalpingeal mucosa.

Hospital records were summarized. The data obtained from these records included age, menstrual history, parity, affected side, race, and hospital status of the patients involved. This was done only in an attempt to compare our series with others presented in the literature.

Originally we tabulated evidences of fibrosis and inflammatory cell infiltration of all 3 layers of the tube, the mucosa, muscularis, and serosa, plus adhesions between the plical folds of the mucosa. Early in our study it was concluded that the only valid criterion was that of adhesions between plical folds, or the formation of glandlike or follicle-like spaces, therefore, this criterion alone was used in our series.

Herzog[19] stated in 1900 that one of the most constant pathologic findings in tubal pregnancy is the presence of polynuclear leukocytes, lymphocytes, and occasionally plasma cells in the muscular, subperitoneal layers and mucosa. Gardner reiterates these findings of inflammation caused by tubal pregnancies. Of the 16 authors quoted earlier who did microscopic analysis, only 7 listed their criterion.[7, 12, 15, 18, 39, 47, 49] They were unanimous in employing plical adhesions (follicular salpingitis) as their evidence of past infection.

## Findings

One hundred and fifteen clinical charts were available for study, the race and hospital status of these patients are seen in Table II. As seen, this series includes a large majority of white private patients.

The average age was 29 years, the youngest patient being 18 years, the oldest 52 years. Fifty-two per cent were nulliparous, 27 per cent primiparous, and the remaining, multiparous. The most productive patient in the series was a para X. The average duration between the last menstrual period and the time of operation was 58.6 days. The right

**Table II.** Hospital status of patients

| | *Private patients* | *Service patients* |
|---|---|---|
| White | 89 | 5 |
| Negro | 11 | 10 |

Table III. Usual findings in patients with ectopic pregnancy

| *Author* | *Age* | *Race* | *Parity* | *Period of amenorrhea* | *Side* |
|---|---|---|---|---|---|
| Ware and Winston[45] | 25 to 30 years | 76 per cent Negro | 0 | 8 to 12 weeks (45.9 per cent) | |
| Beacham and associates[4] | 26 to 35 years | | 1 | 2 months | Right (60.1 per cent) |

tube was affected in 53 per cent of the cases, the remaining pregnancies were in the left tube.

Table III gives the data from two recent articles; it is seen there is good agreement. We feel the only possible significant distortion of our group of patients from other series reported in the literature might lie in the relative high frequency of white to Negro patients.

Microscopically, in our cases, evidence of some stage of inflammation was present in practically every slide viewed. Acute reactions were seen with rugae being swollen and bloated with edema, leukocytic infiltration of all layers, blood vessels engorged, and leukocytes and desquamated epithelial cells in the lumen. Subacute to chronic changes were evidenced by lymphocytes and plasma cell infiltration of all layers, diminution of edema, and return of blood vessels to normal size. In no instance did we see extensive fibrosis of the tubal wall. Very often the evidence, as stated above, of both acute and chronic reactions was seen on the same section, the various cellular types being intermingled. All of these reactions listed above, we feel, were a result of tubal wall response to the ectopic nidation itself. These were not evidence of previous infection; we considered only adhesions between plical folds of mucosa as evidence of this.

There was a great variation in the number of these adhesions and the formation of glandlike or follicle-like spaces. For this reason we have divided this material into mild and severe groups. The characteristics of each group are given in Table IV and are seen in Figs. 1 and 2.

Forty-six of the 121 patients (38 per cent) were found to have residues of previous inflammation of the tubes. Twenty-three were in the mild group and 23 in the severe group.

Two instances of tubal pregnancy were associated with definite tubal endometriosis. In one, however, the blocks were cut in such a manner that relationships were obscure. Intramural and serosal endometriotic areas were present but we are not certain whether endosalpingeal endometriosis was present or whether the implantation site was in an area of endometriosis. In the other instance the endosalpingeal mucosa had apparently been

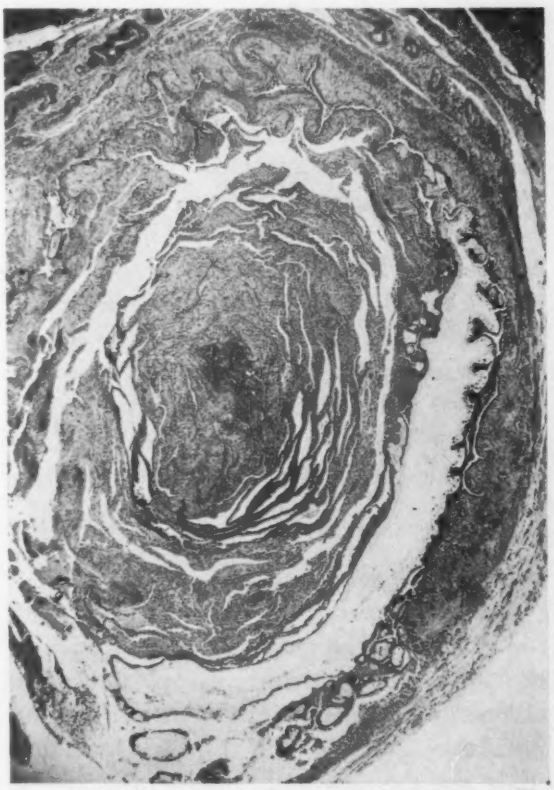
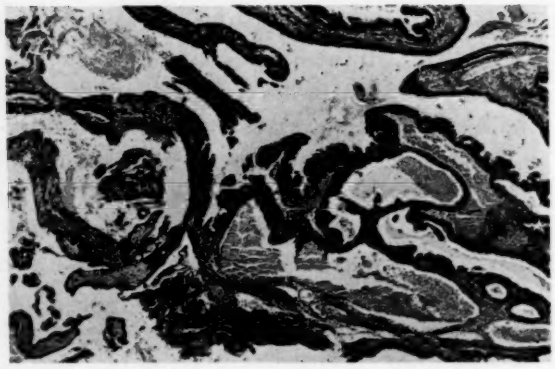


Fig. 3. A tubal pregnancy in which there is endosalpingeal endometriosis. To the left and at the bottom are placental villi. One villus has been cut longitudinally and shows marked hydropic changes. (Original magnification, ×27.)



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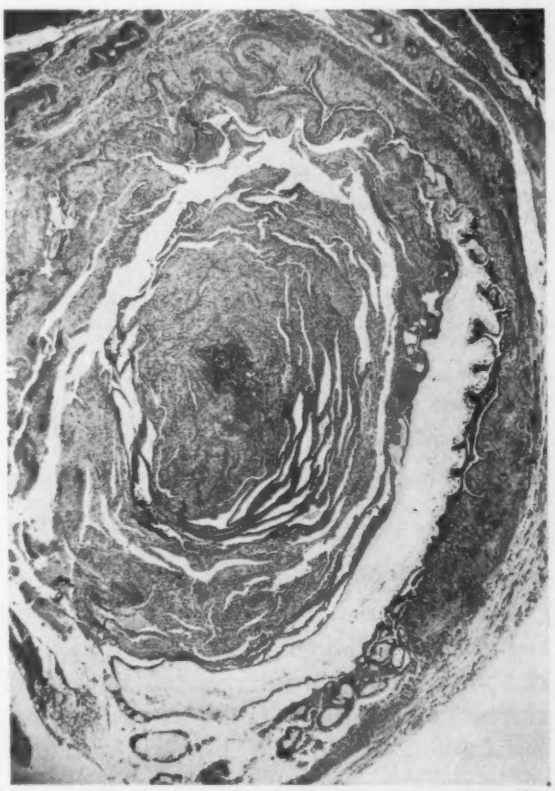

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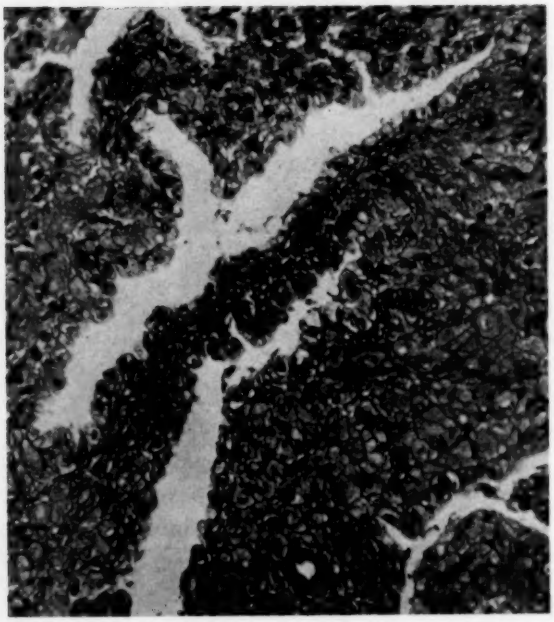

**Fig. 4.** A high-power photomicrograph showing a marked decidual reaction in the ectopic endometrium. The slitlike spaces are endometrial glands with the usual low epithelium of secretory exhaustion. (Original magnification, ×225.)

replaced by endometriosis. Typical endometrium of pregnancy is shown in Figs. 3 and 4, with marked decidual reaction and glands with the usual low epithelium of secretory exhaustion. It seems likely in this case that implantation occurred in an area of endosalpingeal endometriosis.

A decidual reaction in the endosalpingeal mucosa was found in 12 of the 121 cases. It was usually relatively sparse, spotty in distribution, and took place in the stromal cells within or near the base of the plica and would not be confused with areas of endometriosis.

## Conclusions

Other commonly quoted etiological theories for tubal pregnancy have not gained the support that the theory of antecedent chronic inflammation has, and perhaps on justifiable grounds. On the basis of this study and the experience of one of us (R. R. G.), congenital anomalies of the Fallopian tubes have rarely been seen and external migration of the ovum seems like an unusual feat. No intrinsic tumors of the tube were seen in this study, and only two cases of endometriosis of the tube were encountered.

In making a study consisting only of microscopic analysis, we believe we have used the only accurate method to determine the presence of residues of previous inflammation and its etiological importance. By process of elimination the criterion used in this study for determining residues seems the only valid one.

Thirty-eight per cent of the patients showed residues of tubal inflammatory disease. This figure is practically in the middle of the group of the 16 authors who included microscopic analysis. Six of these authors agree with us within 5 per cent. Only 2 of these 6 were among those stating their criteria. Of the remaining 10, 5 stated their criteria. Our difference in results with those not stating criteria could be on the basis of that fact alone. Since the criteria for the remaining 5 articles was apparently the same as ours, the only explanation for differences in results must be either a variation in the material itself or in its interpretation.

**Table IV.** Findings in the mild and severe groups of follicular salpingitis

| *Characteristics* | *Mild group* | *Severe group* |
|---|---|---|
| Location of adhesions | Mainly peripheral | Across entire lumen or greater portion thereof |
| Around entire circumference | Usually | Yes |
| Width of connecting bands | Variable | Usually small |
| Size of gland-like spaces formed | Usually small | Large |
| Definite overall follicular pattern | No | Yes |

Early in this study it was conceded that no single causal factor could be applied to all cases; however, in agreement with most authors it was found that residues of chronic infection in the tube play the major demonstrable role.

## Summary

1. The gravid uterine tubes of 121 patients with ectopic pregnancies have been studied microscopically for evidence of residues of antecedent tubal inflammation.

2. Thirty-eight per cent were found to have such evidence of residues of pelvic inflammatory disease.

3. In at least one instance endosalpingeal endometriosis may have been a factor in the production of tubal pregnancy.

4. Antecedent tubal inflammation is the major demonstrable associated factor and probable cause of tubal pregnancy.

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## Discussion

Dr. J. W. Roddick, Evanston, Illinois. Discussion of this paper is made somewhat difficult by the fact that the authors have very completely reviewed the literature, ably documented their findings, and come to the only possible conclusions from those findings. Unfortunately,

however, by their very criteria, they have limited their study to a search for evidence of previous endosalpingitis rather than for evidence of any previous pelvic infection, as suggested by the title. Fusion of the plica of the uterine tubes is the result of endosalpingitis. Since it is a residue of previous infection rather than a currently active infection, it is more properly termed "follicular hydrosalpinx" than "follicular salpingitis." Whatever one chooses to call it, it is nonetheless a result of previous endosalpingitis only. By using this as their sole criterion for previous infection, the authors have not been able to include residues of infections of the cellulitis type which might not attack the tubal mucosa. One might postulate that a severe infection in the wall of a tube could be followed by enough fibrosis of the wall to cause a loss of the proper propulsive mechanism of the involved tube. Such fibrosis could well be present without concomitant follicular hydrosalpinx and yet be enough to prevent the fertilized ovum from traversing the entire length of the tube, with a resultant ectopic implantation.

Drs. Bone and Greene have rather rapidly dismissed the possibility of excessive fibrosis in the walls of the tubes that they studied. However, it is often very difficult to differentiate fibrous tissue from smooth muscle without employing special techniques. The authors evidently used sections stained only with hematoxylin and eosin and examined only with ordinary light. This suggests that they may be confusing fibrosis in the tube wall with the normal layers of smooth muscle, at least in some instances. If an excess of fibrous tissue could be demonstrated in the wall of the involved tube, this might provide suggestive evidence that impaired motility may also be a factor in the production of tubal pregnancy.

To test this hypothesis, I selected sections from 3 uterine tubes from recent surgical specimens removed at the Evanston Hospital. One of these was from a 32-year-old woman with carcinoma of the breast and was assumed to be normal. The second was from a tube containing an ectopic pregnancy and showing slight follicular hydrosalpinx. The last was from a 40-year-old patient with a clinical and gross diagnosis of residues of pelvic inflammatory disease; the mucosal folds were not involved. In the available time I was unable to get any special stains on these specimens. Therefore, I made use of the positive uniaxial birefringence of collagen which allows it to be seen under polarized light, while smooth muscle does not show up at ordinary magnification. While the myofibrils of smooth muscle are birefringent, they are very fine and can be seen only under very high magnifications. Using this technique, I was able to demonstrate what I consider to be an increase in the fibrous connective tissue in the wall of the diseased tubes when compared to that of the normal. These findings can be seen in the slides, comparing ordinary stained section with the same area when viewed with polarized light. I interpret these findings to indicate a relative increase in collagen in the walls of the tubes which have been involved in pelvic inflammatory disease.

I will readily admit that these 3 specimens, in themselves, prove nothing. I do believe, however, that they indicate the possibility that there may well be something other than follicular hydrosalpinx involved in the etiology of tubal pregnancy in those cases where previous infection is a factor. Perhaps a study along these lines will show that more than 38 per cent of the tubes harboring ectopic gestation have evidence of previous infection.

DR. GREENE (Closing). Dr. Roddick, you did not control your study very well. Your section showing less collagen is from the outer portion of the tube, while the one showing more collagen is from an area nearer to the uterus. Perhaps there is a normal variation in the amount of collagen in the two areas.

# Interstitial pregnancy following homolateral salpingectomy

## A report of 6 new cases and review of the literature

JOHN W. SIMPSON, M.D.*
CHARLES D. ALFORD, M.D.
ALEXANDRE C. MILLER, M.D.
*New Orleans, Louisiana*

THE occurrence of interstitial ectopic pregnancy at the site of a previous salpingectomy is a rare and often dramatic phenomenon. It may follow any known type salpingectomy including that with cornual resection. Because one of us (J. W. S.) was recently confronted with an instance of this unusual condition (Case 5) and dealt indirectly with another (Case 3), we were prompted to review the records of the Charity Hospital at New Orleans for interstitial pregnancies following prior homolateral salpingectomy. From 1942 to the present we found 6 cases. During this period 170,622 babies were delivered and approximately 1,400 ectopic pregnancies were treated at this institution. Each of the 6 patients in the following case reports had all deliveries and surgical procedures here, thus furnishing complete records and descriptions all in one chart.

It is generally held that carefully performed wide cornual resection will prevent subsequent homolateral ectopic pregnancy. However, this concept is still debated in the recent literature. The purpose of this paper is to reassess the merit of cornual resection when performed as prophylaxis against recurrent homolateral ectopic pregnancy. From our study of 46 cases it would appear that simple salpingectomy is almost equally effective prophylactically, is rapidly and easily performed, and is never associated with future calamitous sequelae such as rupture of the gravid uterus through an old cornual scar.

*From the Obstetrical and Gynecological Service, Independent Unit, Charity Hospital.*

**Present address: United States Army Hospital, Camp Wolters, Texas.*

**Case 1.** Mrs. A. J., a 26-year-old para 5-1-4, was admitted to the hospital on Feb. 24, 1952, with a chief complaint of "cramping stomach pains" for 3 days, much more severe on the day of admission. She also complained of dysuria, nausea, and vomiting. The last normal menses had commenced about 6 weeks previously. On physical examination the blood pressure was normal and the pulse rate was 100. The abdomen was distended and dull to percussion but with only moderate tenderness and guarding. Bowel sounds were present but hypoactive. Bimanual examination of the pelvis revealed fullness in the cul-de-sac and a tender uterus which could not be well outlined. The adnexa were not felt as such and the cervix appeared cyanotic. There was no vaginal bleeding. Pelvicentesis produced 20 c.c. of dark, nonclotting blood. Urinalysis was negative and the hematocrit determination was 29 per cent.

Review of this patient's past records revealed right salpingo-oophorectomy without cornual resection for ectopic pregnancy on Dec. 13, 1949. This was followed by one normal vaginal delivery.

Immediate laparotomy revealed about 1,500 c.c. of intraperitoneal blood and a bleeding rent

in the right uterine cornu. The right tube and ovary were absent. The left adnexa appeared normal. A right cornual resection was performed. The patient withstood the procedure well and was discharged on the eighth hospital day after an uneventful postoperative course.

The pathologist reported "tubal pregnancy, chorionic villi and decidua present" on microscopic examination. His description of the gross specimen was "an irregularly shaped piece of grayish white tissue with one recent blood clot covering one surface."

Subsequent to her second homolateral ectopic pregnancy this patient was uneventfully delivered of 4 full-term infants, had one miscarriage, and when last seen in the clinic on Jan. 22, 1960, was again pregnant.

**Case 2.** Mrs. C. P., a 42-year-old para 14-1-9, was admitted to the hospital on Nov. 18, 1952, with a chief complaint of "pain in the stomach" for about 12 hours. The pain was described as severe, sudden in onset, associated with rectal tenesmus, and followed by several episodes of fainting. Shoulder pain was present but there was no vomiting and no vaginal bleeding. Last normal menses had occurred about 6 weeks previously. Physical examination revealed a blood pressure of 90/60, pulse rate of 100, and normal temperature. The abdomen was tender with rebound tenderness present, most marked in the right lower quadrant. The cervix was bluish and tender to manipulation. The uterus and adnexa were felt to be normal by the examining physician. Pelvicentesis produced 15 c.c. of dark, nonclotting blood. Urinalysis was negative and the hematocrit was 27 per cent.

Review of previous records showed right salpingo-oophorectomy without cornual resection for right oviductal pregnancy in 1944.

At laparotomy 1,500 to 2,000 c.c. of blood was found in the abdomen. The source of hemorrhage was noted to be at the right uterine cornu. The right tube and ovary were absent. Whole blood was transfused directly into the right common iliac artery to combat profound shock. A right cornual resection was performed. No abnormality of the left adnexa was noted. The patient did well postoperatively and was discharged on the ninth hospital day.

The pathologist described grossly a pyramidal wedge of tissue 4 by 3 cm. The microscopic description was: uterine wall showing proliferative endometrium: Fallopian tube containing decidua and fragments of placenta.

Following this admission the patient's only known pregnancy resulted in complete abortion in November, 1953.

**Case 3.** Mrs. M. M., a 23-year-old para 1-1-0 (ectopic pregnancy Feb. 14, 1956), was admitted to the hospital on July 13, 1957, with a chief complaint of sudden onset of severe generalized abdominal pain about 2 hours prior to admission. She also complained of vomiting and fainting. Last normal menses had occurred 2 months previously. Significant physical findings consisted of blood pressure 100/60, pulse 134, abdominal tenderness and rebound tenderness most marked in the right lower quadrant, and absent bowel sounds. The uterus and adnexa were not outlined because of guarding. There was no vaginal bleeding. Pelvicentesis was productive of 3 c.c. of dark, nonclotting blood. Urinalysis was negative and the hematocrit determination was 28 per cent.

Past history and review of previous records revealed right salpingectomy with cornual resection on Feb. 14, 1956, for ectopic pregnancy.

At laparotomy an estimated 2,000 to 2,500 c.c. of clotted and nonclotted blood was found. The origin of hemorrhage was seen to be a large, irregular defect in the right cornual portion of the uterus. The right ovary and the round and broad ligaments were intimately adherent to this area. There were many old pelvic adhesions involving mainly uterus and small bowel. The left adnexa appeared normal except for tuboovarian adhesions. A right oophorectomy and cornual resection were performed. The area was peritonized. The postoperative course was complicated by adynamic ileus and high fever. At operation the patient had inadvertently received 1,000 c.c. type O, Rh-positive blood (her blood was type O, Rh negative). Therapy was successful and she was discharged asymptomatic on the twelfth hospital day.

The pathologist reported ovary with corpus luteum of pregnancy and oviduct with ectopic pregnancy and hematoma formation.

About 16 months after the second right-sided ectopic pregnancy this patient developed a pregnancy in the left tube which ruptured on Dec. 26, 1958. Total abdominal hysterectomy and left salpingectomy were performed by one of us.

**Case 4.** Mrs. M. V., a 34-year-old para 8-2-4, was admitted to the hospital on June 18, 1954, with a chief complaint of "stomach pain" of several days' duration. Aside from a feeling of faintness, there were no other complaints. She

had missed no menstrual periods. The patient volunteered that she had had an ectopic pregnancy 7 years earlier and believed that she was again "pregnant in the tube." Physical examination revealed a blood pressure of 92/76, pulse rate of 116, generalized abdominal tenderness, and rebound tenderness most marked in the right lower quadrant. Bowel sounds were recorded as normal. The cervix was soft and cyanotic. The uterus was 4 to 6 weeks gestational size. Palpation of the adnexa revealed tenderness and an indistinct mass on the right. Pelvicentesis produced dark, nonclotting blood. Urinalysis was negative and the hematocrit determination was 29 per cent.

Past history and review of operative dictation records described prior right salpingo-oophorectomy with cornual resection for ectopic pregnancy on March 23, 1951. This was followed by three normal vaginal deliveries.

At laparotomy the abdomen was found to contain 2,000 to 2,500 c.c. of blood and clots. Further exploration revealed a defect in the right cornual portion of the uterus which was soft and friable and entered the myometrium to a depth of 1 cm. This was interpreted as a ruptured right interstitial pregnancy. The right tube and ovary were absent. The left adnexa appeared normal. A total abdominal hysterectomy was performed. The postoperative course was complicated by oliguria and intravascular hemolysis secondary to incompatible blood transfusion. Recovery was rapid and the patient was discharged on the fifteenth hospital day.

The pathologist's report showed "chronic cervicitis with squamous metaplasia, endometrium with decidual reaction of pregnancy and slight hypertrophy of the myometrium. The fetus and membranes were inadvertently discarded but gross interpretation suggests that implantation was in the region of the right cornu but not interstitial."

This case is included as being interstitial even though the pathologist noted a suggestion of doubt. The surgeon based his opinion on an entire specimen in situ, an advantage which the pathologist did not have.

Case 5. Mrs. M. W., a 29-year-old para 1-0-1, was admitted to the hospital on March 6, 1959, complaining of "crampy pain" in the lower abdomen for about 6 days. The pain had become much worse on the day of admission and was associated with nausea, dysuria, and weakness. The patient had missed three menstrual periods. Positive physical findings consisted of blood pressure 100/70, pulse rate 120, pallid mucous membranes, abdominal tenderness, and rebound tenderness most marked in the right lower quadrant. Bowel sounds were absent. The uterus was 6 weeks gestational size, and a soft, indistinct bulge in the right adnexa was noted. Pelvicentesis was productive of a copious amount of dark, nonclotting blood. Urinalysis was negative and the hematocrit determination was 18 per cent.

Past history and review of previous records revealed a right salpingo-oophorectomy on Dec. 8, 1948, for torsion of a right ovarian cyst. This was described as total salpingectomy with careful peritonization but no cornual resection. Ten years later the patient was delivered of a term pregnancy complicated by amnionitis and endometritis secondary to premature rupture of the membranes.

At laparotomy the abdomen was emptied of 1,500 to 2,000 c.c. of blood. A large blown-out area involving most of the right upper quadrant of the uterus was bleeding freely. A 3 to 4 month size fetus was found between the leaves of the broad ligament. The right adnexa were absent. Multiple old pelvic adhesions and a left hydrosalpinx were present. A total abdominal hysterectomy, left salpingectomy, and resection of a corpus luteum hematoma from the left ovary were performed. The patient withstood the procedure well and had a benign postoperative course except for a low-grade wound infection. She was discharged on the tenth hospital day.

The pathologist reported chronic cervicitis, endometrium with marked decidual reaction, interstitial remnant of the right oviduct showing decidual reaction and degenerating chorionic villi, left oviduct with acute and chronic salpingitis, placenta, and a somewhat macerated fetus measuring 8 cm. Unfortunately, no report was returned on the tissue removed from the left ovary. Grossly this appeared to be a corpus luteum of pregnancy.

Case 6. Mrs. J. F., a 27-year-old para 2-2-0, was admitted to the hospital on March 21, 1960, with a chief complaint of cramping lower abdominal pain of 24 hours' duration. She had also experienced recent vomiting and fainting. Last normal menses had occurred 2 months previously. Physical examination revealed blood pressure 70/40 and pulse rate 120 per minute. Considerable pallor was noted by the examiner. The abdomen was slightly distended and generally tender; bowel sounds were hypoactive. On

pelvic examination the cervix appeared slightly blue and a small erosion was present. The uterus was somewhat enlarged and tender to manipulation. No adnexal masses were palpable. Pelvicentesis produced 4 c.c. of nonclotting blood. The hematocrit determination was 30 per cent and urinalysis was negative.

Past history revealed acute pelvic inflammatory disease in 1952, late complete abortion of a stillborn monster on Nov. 20, 1958, and a right salpingectomy with cornual resection for ruptured ectopic pregnancy on July 29, 1959.

At operation about 2,000 c.c. of blood was removed from the abdomen by suction and sponging. Exploration revealed a 6 cm. fetus free in the cul-de-sac and a rent in the right cornual area of the uterus. The right tube was absent. A total abdominal hysterectomy was performed. The patient did well postoperatively except for some patchy atelectasis of the right lung. She was discharged on the ninth hospital day. Two days later she was treated in the outpatient department for a small wound hematoma which had opened and drained spontaneously. On subsequent clinic visits no abnormalities were noted.

The pathologist described the gross specimen as "uterus and cervix with a cavity in the right cornual area filled with tissue fragments and blood clots and measuring 6 by 3 cm. Fetus, crown-rump measurement 7 cm." Microscopically he described "uterus, gestational; cervix, gestational; placental fragments."

### Review of the literature

We were able to collect 40 cases (Table I) from the literature. Hofmeier[29] first reported this entity in 1905. Morfit's[36] earlier case (1900) occurred after only partial salpingectomy. There were 36 cases[9, 11, 22] in the literature following partial salpingectomy. These in our opinion represent a somewhat different situation since recanalization of tubal stumps is a well-recognized phenomenon and an incidence of 2 to 4 per cent[17] interstitial implantation would be anticipated in any subsequent ectopic pregnancy whether the tube were partially excised or ligated or a tuboplasty performed. When only a short stump remains, it may at times be obscure as to whether implantation were truly interstitial or not. For these reasons and for clarity in considering the possible prophylactic merits of cornual resection as opposed to simple salpingectomy alone, we will limit our discussion to interstitial pregnancy following these two procedures only. Note must be taken of one other case, that of Andrews[1] in 1912, in which congenital absence of the homolateral tube and ovary must be assumed since the author offers no other explanation. This case is of special interest when mechanism of implantation is considered.

There are three or four theories[5, 34] concerning the mechanism of implantation. The majority of surgeons dealing with this condition felt that recanalization had occurred at the site of previous salpingectomy. This is possible even after cornual resection.[12, 30] The ovum may gain access to these recanalized tubes via the peritoneal cavity either from the homolateral ovary or, in its absence, from the opposite ovary by transmigration. While external transmigration is the rule and may occur in as many as 11.4 per cent[41] of tubal pregnancies in general, there are at least 3 cases[4, 5, 20] in which internal transmigration appears well substantiated. Probably no other route was available in Andrews' case.[1] In the event of internal transmigration only partial recanalization would suffice since the ovum makes its way down the contralateral tube and across the endometrial cavity to implant in the wall of the opposite cornu. In most of these 40 cases fertilization probably took place in the peritoneal cavity or in the recanalized tube itself.

Frankel's[21] 4 cases constituted the longest single series. Conley and Klieger[10] and Fulsher[22] each reported one death, giving a mortality rate of 5 per cent in the condition under discussion. This is about twice that of ectopic pregnancy in general.[15] The most common indication for the prior salpingectomy was an earlier ectopic pregnancy, this being noted 24 times or 60 per cent of the total number reported. It has been estimated that ectopic pregnancy will recur in 4 to 5 per cent[34] of women again able to conceive. The age range in these 40 cases was 21 to 36 years. The interval between salpingectomy and subsequent homolateral interstitial pregnancy varied from 6 months to

Table I

| Case | Author | Date | Age | Indication for previous salpingectomy | Interval | Type of salpingectomy | Duration of gestation |
|---|---|---|---|---|---|---|---|
| 1 | Hofmeier[29] | 1905 | 28 | Ectopic | 6 months | Simple salpingectomy | 3 to 4 weeks |
| 2 | Campbell[7] | 1910 | 30 | Unknown | 6 years | Simple salpingectomy | 10 weeks |
| 3 | Nacke[37] | 1911 | | Sterilization | 3 months | Cornual resection | 8 weeks |
| 4 | Benzel[3] | 1912 | 30 | Ectopic | 1 year | Simple salpingectomy | 8 weeks |
| 5 | Guidal[26] | 1918 | 35 | Ectopic | 5 years | Simple salpingectomy | 6 weeks |
| 6 | Douglas[16] | 1920 | 30 | Ectopic | 2 years | Simple salpingectomy | 4 weeks |
| 7 | Guibe[25] | 1928 | 29 | Ectopic | 1 year | Simple salpingectomy | 6 weeks |
| 8 | Guibe[25] | 1928 | 31 | Ectopic | 3 years | Simple salpingectomy | 6 weeks |
| 9 | Chabrut[8] | 1929 | 24 | Ectopic | 5 months | Simple salpingectomy | 4 weeks |
| 10 | Chabrut[8] | 1929 | | Ectopic | 2 years | Cornual resection | 4 weeks |
| 11 | Held[28] | 1930 | 31 | Ectopic | 4 years | Cornual resection | 3 months |
| 12 | von Schroeder[43] | 1934 | 21 | Ectopic | 3 months | Cornual resection | 8 weeks |
| 13 | Deutsch[13] | 1935 | 27 | Ectopic | 2 years | Simple salpingectomy | 7 weeks |
| 14 | Meyer[35] | 1935 | 31 | Ectopic | 6 years | Simple salpingectomy | 4 weeks |
| 15 | D'Errico[12] | 1937 | 29 | Pelvic abscess | 8 years | Simple salpingectomy | 5 weeks |
| 16 | Batiztalvy[2] | 1938 | 26 | Ovarian cyst | 7 months | Simple salpingectomy | 4 weeks |
| 17 | Forman[20] | 1939 | 31 | Ectopic | 3 years | Simple salpingectomy | 3 months |
| 18 | von Vegh[44] | 1940 | 36 | Intraligamentous cyst | 13 years | Cornual resection | 8 weeks |
| 19 | Cabrera[6] | 1945 | 28 | Ectopic | 1 year | Simple salpingectomy | 8 weeks |
| 20 | Cabrera[6] | 1945 | 30 | Dermoid cyst | 1 year | Simple salpingectomy | 6 weeks |
| 21 | Gee[24] | 1945 | 35 | Ectopic | 11 years | Simple salpingectomy | 6 weeks |
| 22 | Doeller[14] | 1946 | 30 | Ectopic | 4 years | Simple salpingectomy | 4 months |
| 23 | Buerger[5] | 1948 | 23 | Tuboovarian abscess | 1 year | Simple salpingectomy | 7 weeks |
| 24 | Frankel[21] | 1948 | 28 | Hydrosalpinx | 5 years | Simple salpingectomy | 8 weeks |
| 25 | Frankel[21] | 1948 | 29 | Unknown | 9 years | Simple salpingectomy | Unknown |
| 26 | Frankel[21] | 1948 | 32 | Ectopic | 1½ years | Cornual resection | 6 weeks |
| 27 | Frankel[21] | 1948 | 28 | Unknown | 3 years | Simple salpingectomy | 3 months |
| 28 | Lingenfelder[34] | 1950 | 30 | Ectopic | 8 years | Simple salpingectomy | 4 weeks |
| 29 | Fairbanks[18] | 1951 | 31 | Cystic ovary | 9 months | Cornual resection | 8 weeks |
| 30 | Leverton[33] | 1952 | 29 | Chronic pelvic inflammatory disease | 6 years | Simple salpingectomy | 8 weeks |
| 31 | Ladas[32] | 1955 | 34 | Ectopic | 10 years | Simple salpingectomy | 6 weeks |
| 32 | Gabriels[23] | 1956 | 24 | Pyosalpinx | 1 year | Cornual resection | Unknown |
| 33 | Speck[40] | 1956 | 24 | Ectopic | 1 year | Simple salpingectomy | 2 weeks |
| 34 | Steadman[42] | 1956 | | Ectopic | 1 year | Simple salpingectomy | Unknown |
| 35 | Conley[10] | 1957 | 31 | Ectopic | 3 years | Simple salpingectomy | 5 months |
| 36 | Conley[10] | 1957 | 22 | Ectopic | 2 years | Cornual resection | 5 weeks |
| 37 | Bickerstaff[4] | 1957 | 30 | Endometriosis | 6 years | Cornual resection | 6 to 8 weeks |
| 38 | Fulsher[22] | 1959 | 26 | Unknown | | Simple salpingectomy | Unknown |
| 39 | Fulsher[22] | 1959 | 30 | Ectopic | 9 years | Simple salpingectomy | 3 months |
| 40 | Fulsher[22] | 1959 | | Ectopic | 3 years | Simple salpingectomy | 5 weeks |

13 years. Apparently, normal vaginal delivery is not too uncommon in this interval but up until Case 1 only 5[5, 22, 42, 43] infants were carried to term by women having had two homolateral ectopic pregnancies. One individual was delivered of 2 of these infants about one year apart without maternal complications. Another was delivered by cesarean section. The remaining 2 sustained either complete or partial uterine rupture requiring hysterectomy. On this basis it would appear that further childbearing in this group of patients is hazardous. Cesarean section may well be indicated as the safest mode of delivery.

Guibe,[25] Chabrut,[8] and Conley and Klieger[10] expressed doubt as to the efficacy of cornual resection for prophylaxis against recurrent homolateral interstitial ectopic pregnancy. Fulsher,[22] in a recent report of sev-

eral new cases and excellent review of the literature, said that cornual resection should be done in all cases where the condition of the patient renders it safe. In this he reiterates what is probably the majority opinion.

There are in the literature 10 reports of homolateral interstitial pregnancy following salpingectomy with cornual resection. To these we add 3 new cases. In addition there are 30 cases following simple salpingectomy alone, to which we add 3 more cases.

## Comment

The 6 cases reported herein are all quite similar and give a representative picture of the ruptured ectopic pregnancy. They serve again to emphasize the value of pelvicentesis. This is particularly true since in no case was there vaginal bleeding or any history of it. We have noted this absence of vaginal bleeding in some other interstitial pregnancies of our experience. Ordinarily vaginal bleeding would be anticipated in 75 per cent[17] of ruptured ectopic pregnancies. The fact that all these cases were on the right side is coincidental. In keeping with other reports, the amount of hemorrhage was excessive. Case 2 illustrates the heroic measures sometimes necessary to combat vascular collapse in this condition. Case 3 is unique in several ways. The patient had 3 ectopic pregnancies in rapid succession and no normal pregnancies. In one a homolateral interstitial pregnancy followed a cornual resection. This case is the only one of these in which transmigration of the ovum did not occur since the pathologist reported a corpus luteum of pregnancy in the homolateral ovary. We believe that transmigration was external in the other 5 cases since there was no evidence to suggest that the ovum had taken the internal route across the interior of the uterine cavity. The tube recanalized in each of these cases with the possible exception of Case 4. From the pathologist's description of the gross specimen this may support Buerger's[5] theory of a fistulous opening in the area of the cornual scar.

From these 6 cases and those in the literature it would appear that recanalization of the tube is the principal factor involved in this rare condition. Fulsher[22] quotes Hasselblatt[27] and Keller[31] as stating that this can result from any small nidus of tubal epithelium left in the region of the scar. This concept seems valid since recanalization after cornual resection has been reported twelve and possibly thirteen times thus far. For cornual resection to be truly effective prophylaxis, all such nests of tubal epithelium would have to be included in the excised tissue. It is not unusual to find spots of tubal epithelium in the endometrium,[38] and their incidence probably increases as the cornual regions are approached. Even the creation of a very large defect in the uterine wall would not insure against recanalization. There are in the literature at least 9 recorded instances[9, 19, 22, 30, 43] of rupture of the gravid uterus at the site of a defective cornual scar. These 9 undesirable sequelae combined with the 13 known failures results in a figure comparable to the 33 homolateral ectopic gestations following simple salpingectomy alone. It is also likely that many more simple salpingectomies have been done. At laparotomy for ectopic pregnancy a wide cornual resection is apt to be bloody and apt to prolong the operating time. Recanalization is also more common in the face of a pregnancy. While no really valid conclusion can be drawn from a study of only 46 cases, in our opinion simple salpingectomy with careful peritonization is preferable to cornual resection and especially when dealing with ruptured tubal pregnancy.

## Summary

1. The literature is reviewed and 40 cases of interstitial pregnancy following homolateral salpingectomy found.

2. Six new cases are reported.

3. Mechanism of implantation is discussed.

4. The prophylactic merits of cornual resection versus simple salpingectomy alone are discussed.

## Conclusions

1. Recanalization of the tubal remnant is the most important factor in this rare condition.

2. External transmigration of the ovum is common.

3. Cornual resection holds no particular advantage over simple salpingectomy with careful peritonization in the prevention of interstitial pregnancy following homolateral salpingectomy.

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# Review of transverse lie at the Methodist Hospital, Brooklyn, 1924-1958

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TRANSVERSE lie is an unusual and dangerous complication of pregnancy. We have selected the term "transverse lie" because it is the one which most clinicians use today and because it seems the most logical one. To many obstetricians the treatment of transverse lie presents no problem, once the diagnosis has been made. Cesarean section has been a well-established operative procedure for the past 25 years. At the Methodist Hospital, Brooklyn, we have obstetrical records available for the past 35 years and we felt that a review of this many cases of transverse lie might answer 2 questions:

1. How has the method of treatment of this condition changed during that time?
2. Can we improve our results, in the interests of both mother and baby?[7]

During the 35 years from 1924 to 1958, there were 91 cases of transverse lie in single pregnancies in 71,897 consecutive deliveries. This represents an incidence of about 1 in 1,200.

Fig. 1 telescopes the history of this complication. It shows the relationships of maternal deaths, fetal survival, cesarean section, and version and extraction. It is obvious from Fig. 1 that the percentage of deliveries by version and extraction has rapidly decreased to about 10 per cent while, conversely, the incidence of cesarean section has reached the 90 per cent level. At the same time the maternal mortality has been reduced to zero with improvement in fetal survival. Additional comments concerning these factors will be made later in the paper.

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### Incidence

As seen in Fig. 2, the incidence of transverse lie in our hospital has decreased while the number of deliveries has increased. We feel that this decrease in incidence is the result of 3 factors: (1) fewer problem cases referred by general practitioners who did home deliveries in the earlier years, (2) the discontinuance of our ambulance service 5 years ago, and (3) the increased use of external version during the last 10 weeks of pregnancy.

The incidence of this complication on the University Service of the Kings County Hospital between 1951 and 1957 has been reviewed for comparison.[9] Table I shows the distribution of 85 cases of transverse lie in 31,356 deliveries, which gives an incidence of 2.7 cases per 1,000 deliveries. This higher incidence, which is about three times that at the Methodist Hospital is due, we think, to the large ambulance district covered by the Kings County Hospital. This higher incidence has been found in many other large series.[1-4, 7, 10, 13-15, 17]

### Parity

It is commonly stated that multiparity is a factor predisposing to transverse lie.[1-8, 10, 11, 14, 16] Our experience has not substantiated this concept completely. As seen in Fig. 3, the fact that the patient is parous seems more important than the degree of her parity. The graph shows that approximately 8 per cent of the patients with transverse lie were primiparas, while in our general obstetric population about 50 per cent of the patients were primiparas. Thereafter, the two curves are essentially identical, right up to and including parity of 12. High parity, however, is a factor to be considered from the standpoint of rupture of the uterus if internal version is done.

### Etiological factors

As might be anticipated, a variety of factors which would interfere with the usual longitudinal lie of a fetus are present in cases of transverse lie.

Fig. 4 shows that placenta previa occurs in 11 per cent; congenital abnormalities of the uterus in 4.4 per cent; fibromyomata uteri in 4.4 per cent; contracted pelvis in 1.1 per cent. There was no known cause in 79.1 per cent. Stevenson,[14] in a report from the Boston Lying-in Hospital, has perhaps the most logical explanation for this group. In a well-executed and controlled roentgenographic survey of 52 cases of transverse lie during the last 10 weeks of pregnancy, he found the placenta in the general fundal region in 48.2 per cent and partly or entirely in the lower uterine segment (including placenta previa) in 44.2 per cent. This location of the placenta naturally would decrease the longitudinal length of the amniotic sac cavity. He concluded, "The basic point our study has shown is that the position of the placenta, in situ in the near-term uterus, has a definite influencing effect upon the presentation of the fetus in an appreciable proportion of cases."

Of the 92.3 per cent of the 52 cases of transverse lie, 36.5 per cent persisted to labor; 11.5 per cent rotated spontaneously to a breech presentation; and 52 per cent spontaneously converted to a cephalic presentation.

### Complications and various other factors associated with transverse lie

Table II shows some of the most frequently occurring complications found in both the Methodist Hospital and the Kings County Hospital series. Placenta previa, congenital abnormalities of the uterus, previous abnormal presentation, rupture of the uterus, prolapse of the cord, and prematurity (35 to 40 per cent) are some of the more common ones. In the Kings County Hospital group there is a higher incidence of fibro-

**Table I.** The incidence of transverse lie at the Kings County Hospital (University Service)

| *Year* | *Cases* | *Deliveries* | *Incidence per 1,000 deliveries* |
|---|---|---|---|
| 1951 | 4 | 2,030 | 1.9 |
| 1952 | 8 | 2,094 | 3.8 |
| 1953 | 8 | 4,256* | 1.8 |
| 1954 | 15 | 4,863 | 3.0 |
| 1955 | 16 | 5,411 | 2.9 |
| 1956 | 19 | 6,205 | 3.0 |
| 1957 | 15 | 6,497 | 2.3 |
| Total | 85 | 31,356 | 2.7 |

*From 1953 on, both divisions of the University Service were included.

**Table II.** Various factors associated with transverse and shoulder presentation

| | *Methodist Hospital* | *Kings County Hospital* |
|---|---|---|
| Placenta previa | 11 | 16.5 |
| Fibromyomas | 4.4 | 8.3 |
| Contracted pelvis | 1.1 | 4.7 |
| Congenital abnormalities of the uterus | 2.2 | 2.3 |
| Previous abnormal presentation | 8.8 | 10.5 |
| Rupture of uterus | 2.2 | 1.7 |
| Prolapse of cord | 6.7 | 16.5 |
| Para v and over | 25 | 33 |
| Babies over 2,500 grams | 61.5 | 60 |
| Babies, 36 weeks and over | 64.7 | 63.5 |
| Dead on admission (of total fetal mortality) | 10 | 20.6 |

**Table III.** Collected fetal mortality* from the literature by method of delivery of transverse and shoulder presentation

| Author | Period | Mortality associated with: Vaginal delivery (%) | Mortality associated with: Cesarean section (%) |
|---|---|---|---|
| Eastman | 1896 to 1931 | 42 | 0 |
| Harris | 1932 to 1948 | 45 | 8 |
| Johnson | 1906 to 1945 | 43 | 25 |
| Mangone and Kane | 1943 to 1952 | 52 | 9 |
| Garber and Ware | 1933 to 1949 | 90 | 8 |
| Cole and Delany | 1932 to 1946 | 28 | 0 |
| Calkins and Pearce | | 32 | 0 |
| Wilson and associates | 1935 to 1950 | 28.5 | 13.8 |
| | 1951 to 1956 | 25 | 3.2 |
| Kings County | 1951 to 1957 | 30 | 9.5 |
| Methodist Hispital | 1924 to 1958 | 28 | 7.2 |

*Fetal deaths on admission excluded.

myomata uteri, contracted pelvis, prolapse of the cord, and percentage of fetuses dead on admission. This is most likely due to the large ambulance service of that hospital and the district which it serves. As was noted in Fig. 1, the incidence of cesarean section has gradually decreased over the years while that of version and extraction has proportionately decreased.

Table III is a representative comparison of fetal mortality in transverse lie by method of delivery, i.e., mortality associated with vaginal delivery and cesarean section as published by various authors over the years. It can be easily seen that the fetal mortality associated with cesarean section is far lower than that associated with vaginal delivery.

### Maternal mortality

Fig. 1 shows that the only maternal deaths occurred during the first 10 years of our survey, from 1924 to 1933, inclusive. These 3 deaths represent a maternal mortality rate of 7.9 per cent for the first 10 years and an over-all maternal mortality of 3.3 per cent.

The first maternal death occurred in 1924. The patient had been brought in by ambulance. The fetal heart was not heard on admission. The diagnoses of placenta previa, ruptured membranes, impacted shoulder and arm, and contraction ring were made. The baby was delivered after craniotomy and decapitation. The mother died of peritonitis.

The second maternal death occurred in 1933. The mother had placenta previa treated by rupture of the membranes and a bag induction. The infant was delivered by internal version and extraction. The uterus was ruptured and the patient died. The baby lived.

The third maternal death also occurred in 1933. The patient had had a transverse lie with her first pregnancy, terminated by decapitation. She had a recurrent transverse lie and an elective cesarean section was performed. The baby lived but the mother died of paralytic ileus.

From the maternal point of view, cesarean section has become much safer and fortunately we have had no maternal deaths associated with this complication in the past 25 years.

### Fetal mortality

There were 20 fetal deaths in the series, which gives a 21.3 per cent fetal mortality for the entire group. Of these 20 deaths, 13 occurred between 1924 and 1933, which represents a fetal mortality of 34.2 per cent

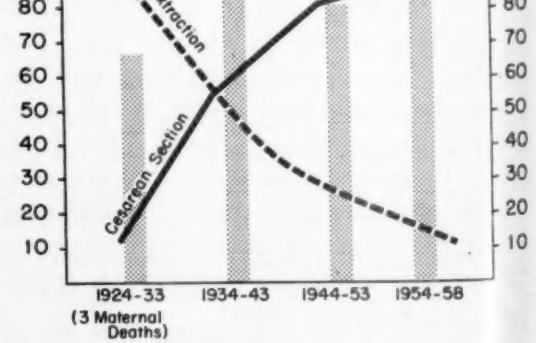


**Fig. 1.** The changing management of transverse lie in relation to maternal deaths and fetal survival.

for this group. Of these 13 deaths, 11 were intrapartum deaths associated with neglected cases and destructive operations. One was due to prematurity and one was associated with placenta previa.

The remaining 7 deaths occurred between 1934 and 1958, for a fetal mortality of 13.2 per cent in the last 25 years. They were due to the following: prolapse of the cord, 1; placenta previa, 1; prematurity, 3; and unknown cause, 2.

The type of uterine incision at cesarean section has also varied as follows: 1920's, only classical incisions; 1930's, one half classical and one half low segment vertical incisions; 1940's, 2 low segment transverse, 5 low segment vertical, and 5 classical incisions; 1950's, 9 low segment transverse, 5 low segment vertical, and no classical incisions.

## Comment

The favorable results reported in this 35 year survey of transverse lie should be interpreted from the standpoint of professional staff and facilities. In 1924, the Obstetrical and Gynecological Department was fortunate in having outstanding specialists for leaders. It was through the efforts of Drs. Paul Humpstone and Ralph Beach that in 1924 a model 80 bed maternity building was opened as a part of a 460 bed general hospital. A cesarean section room was a part of the labor and delivery room suite. An ideal maternity clinic and laboratory was on the ground floor. In addition to this, in 1924, the application of the clinical research of Dr. Harry Mayes[12] on vaginal antisepsis resulted in the lowest maternal mortality reported in this country in that period. Also, a graduate education program was established in the form of a one-year residency in 1928 which was expanded to a 4 year program in 1946. Since the year 1928, new staff privileges were given only to specialists with adequate training. Since 1935 only qualified staff members have had the privileges of the Department. The fruitful results of this foundation are well demonstrated in Fig. 1. It can be seen that no

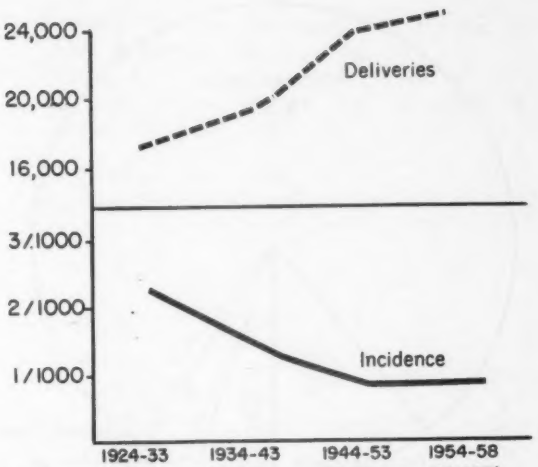


**Fig. 2.** Decreasing incidence of transverse lie compared to the number of deliveries.

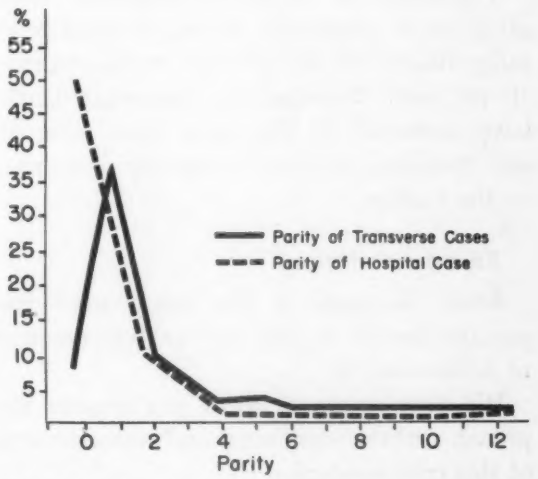


**Fig. 3.** Parity in transverse lie cases compared to parity in hospital deliveries.

maternal deaths have occurred since 1933 and that the fetal survival rate is of the same magnitude for the last 25 years.

## Conclusions

Cesarean section has become a safer operative procedure for the mother. Because of this, since 1934, cesarean section has gradually supplanted version and extraction in the management of transverse lie. However, the fetal survival rate has not increased significantly in the last 25 years although the cesarean section rate has increased from 50 per cent to 90 per cent.

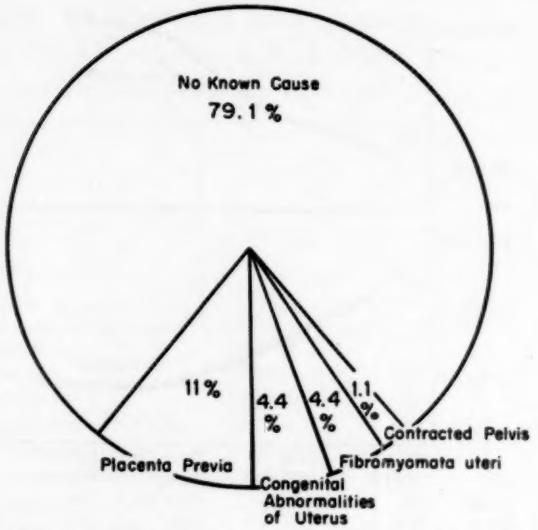


**Fig. 4.** Etiological factors associated with transverse lie.

Transverse lie is still a dangerous complication of pregnancy. It represents a mortality hazard to the fetus of approximately 10 per cent. Although no maternal deaths have occurred in the same time interval, this condition still is intrinsically dangerous to the mother.

### Recommendations

Early diagnosis is the single most important factor in the proper management of transverse lie.

We have outlined below our present approach to the diagnosis and management of this complication.

1. Abdominal examination at each prenatal visit, and especially during the last 10 weeks of pregnancy.

2. External version should be performed antenatally if easily done and if not contraindicated. This is also recommended by many of the authors reviewed.

3. If a transverse lie of a term-sized fetus persists, the patient should be admitted to the hospital.

4. On admission to the hospital, the patient should have an x-ray study of the abdomen to confirm the presentation and to outline the placental site if possible.

5. In our opinion, if the abnormal presentation is confirmed, the patient should be delivered by cesarean section.

6. Those patients who are admitted to the hospital with a history of ruptured membranes prior to admission, in labor or not, should be delivered by cesarean section after the abnormal presentation is confirmed by vaginal examination. We ordinarily do not advise confirmation by x-ray study because of the common delay associated with that procedure and the danger of prolapse of the cord when the patient is out of the maternity service.

7. Those patients who are admitted to the hospital in active labor with intact membranes should have individualized management. Internal version and extraction may be indicated in selected cases. Our criteria for internal version are intact membranes, no contraction ring, complete or nearly complete cervical dilation, and lack of contraindications such as contracted pelvis, placenta previa, and high parity.

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CURRENT OPINION

Clinical problems

# Adenocarcinoma of the endometrium following radiation therapy and associated with hilus cell hyperplasia

**Case presentation**

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*Albert Einstein College of Medicine*

A 65-year-old white widow, gravida iii, para i, was admitted to Sloane Hospital for Women on Nov. 8, 1960, because of painless vaginal bleeding of 5 months' duration. Menarche was at age 18; menstrual cycles were 24 days with flow 3 days. She had a normal term delivery in 1917 at 22 years and spontaneous abortions in 1923 and 1926, both treated by curettage.

In 1932, at the age of 37, she developed hematuria and was treated at Bellevue Hospital with a clinical diagnosis of carcinoma of the bladder. She received 2,800 r air dose through four pelvic ports with a 200 kv. machine, giving an estimated midpelvic dose of 1,350 ± 200 r. Following this treatment menses ceased. Because of recurrent hematuria and passage of gravel, suprapubic cystostomy and curettage of the bladder was done 7 months later. Tissue removed at this time was diagnosed as "infected papilloma of bladder, with ulceration and calcareous encrustation." She remained asymptomatic for 16 years.

In 1948 at the age of 53 she developed weight loss, anemia, anorexia, and a mass in the right side of the abdomen. She was admitted to Presbyterian Hospital where an adenocarcinoma of the cecum was resected and an ileotransverse colostomy was performed. The regional lymph nodes were free of tumor on microscopic examination, and no tumor implants were seen in the pelvis at exploration. She remained in good health until the present admission.

In July, 1960, vaginal staining began, continuing to the time of admission and progressing to frank flowing without clots. She consulted her personal physician late in October and was admitted on Nov. 8, 1960. There were no other complaints on admission. Specifically, she denied hirsutism, change in voice, weight, or body build, alopecia, or acne.

Physical examination was essentially negative. There were no stigmas of androgenization. The breasts were small and atrophic, free from discharge or masses. Abdominal scars consistent with previous surgical procedures were present, and there was a zone of dry, scaly, somewhat pigmented skin over the suprapubic area. Pelvic examination revealed atrophic external genitals consistent with the patient's age. The introitus admitted two fingers tightly, and the vaginal mucosa

was thin, shiny, and delicate. The cervix was clean and small. The uterus was small, anterior, and freely movable. There were no adnexal masses. Preoperative endocrine studies were not done. Exfoliative cytology was Class II with trichomonads; there was no cornification. Other laboratory studies were unrevealing. Dilatation and curettage was done 2 days after entry, and microscopic examination yielded a diagnosis of a well-differentiated adenocarcinoma of the endometrium (Fig. 1). A total abdominal hysterectomy and bilateral salpingo-oophorectomy were done on November 16. She was discharged 10 days later after an uneventful postoperative course. Pelvic irradiation of 4,000 r to the midpelvis by the betatron was scheduled.

**Pathologic observations.**

*Gross.* The uterus was small and atrophic, weighing 60 grams. The exocervix and endocervix were normal. The endometrial cavity contained a fungating necrotic tumor arising from the right cornu. It measured 4 cm. in average diameter and invaded the subjacent myometrium to a depth of 0.5 cm. Elsewhere the myometrium averaged 1.2 cm. in thickness. The Fallopian tubes were thin and atrophic. The right ovary measured 3 by 2 by 2 cm., the left ovary 3.5 by 1.5 by 1.0 cm. Their external surfaces showed normal convolutions, and on section numerous corpora albicantia were seen. No remarkable features were seen in the hila.

*Microscopic.* The endometrium was largely replaced by a well-differentiated overgrowth of neoplastic glands exhibiting the usual cellular features of malignancy. Mucus secretion was demonstrable by the Hotchkiss-McManus-PAS technique. Invasion of lymphatic and venous channels in the subjacent myometrium was readily demonstrable. In areas where neoplastic transformation had not occurred, the endometrium was a thin, atrophic mucosa with simple tubular glands embedded in a compact cellular stroma.

The ovarian cortices were of normal postmenopausal cellularity with no follicular structures, and there were numerous corpora albicantia in the inner cortex and medulla.

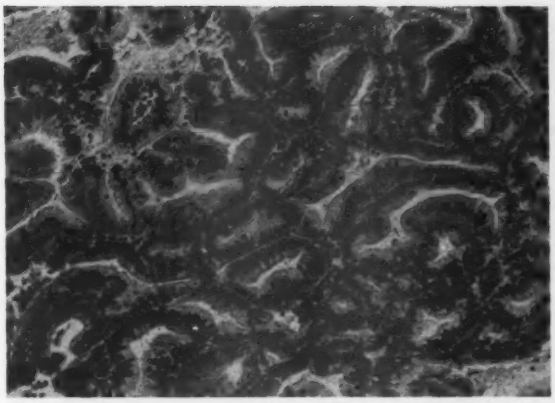

**Fig. 1.** Uterine curettings reveal a well-differentiated adenocarcinoma. (Hematoxylin and eosin. ×100.)

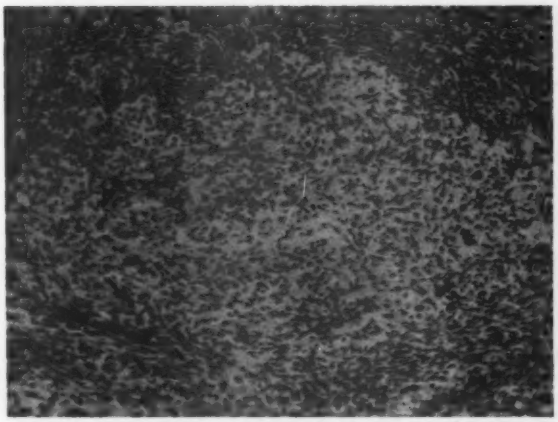

**Fig. 2.** Section of ovary showing an intracortical nest of polyhedral cells with abundant pale-staining cytoplasm. (Hematoxylin and eosin. ×40.)

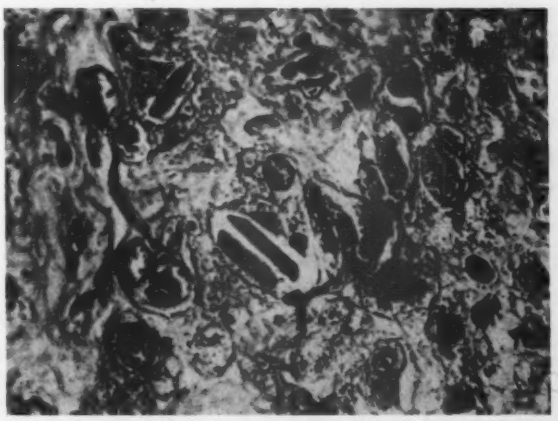

**Fig. 3.** Crystalloids of Reinke are seen clearly in a section overstained with eosin. (Hematoxylin and eosin. ×600.)

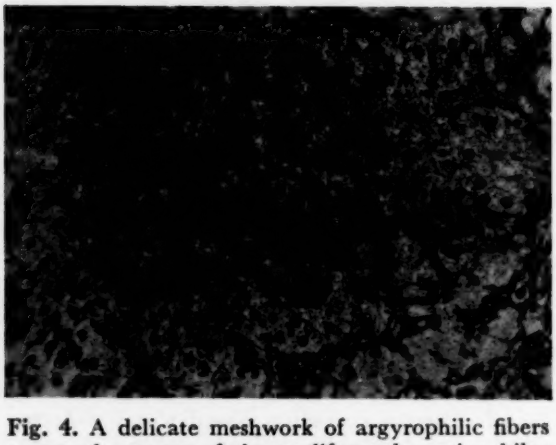

**Fig. 4.** A delicate meshwork of argyrophilic fibers surrounds groups of the proliferated ovarian hilus cells. (Wilder's reticulum stain. ×220.)

An occasional nest of polyhedral cells was found in the cortex (Fig. 2). Such nests were indistinctly marginated, and the polyhedral cells were relatively small, arranged in a mosaic, and conforming in every particular to the cells seen in greater quantity in the region of the hilus.

Occupying a great proportion of the hilar area in both ovaries, somewhat more extensive on the right than on the left, was a diffuse proliferation of large and small nests and cords of polyhedral cells scattered in and among the nerves and blood vessels of the area. Although the proliferation was multifocal and none of the clusters was grossly visible as a discrete mass, some of the larger aggregates approached tumefaction. The largest single cluster measured slightly less than 1 mm. in diameter. The constituent cells were polyhedral, measuring 30 to 40 $\mu$ in diameter, with a centrally placed, relatively unstructured nucleus, sharply defined cell margins, and an eosinophilic cytoplasm that was often abundant but sometimes foamy, even vacuolated. Yellow-brown lipochrome pigment was readily identified in sections counterstained lightly with eosin. Crystalloids of Reinke (Fig. 3) were easily identified in sections stained more heavily with eosin. They stained dark black with Heidenhain's iron hematoxylin and bright orange-red with Masson's trichrome stain. Delicate sinusoidal blood vessels and an inconspicuous collagenous fibrous network formed the stroma for these nests, and sections stained by Wilder's method for reticulum revealed delicate argyrophilic fibrils (Fig. 4) coursing around small groups of these cells. In a few fields typical nests of these polyhedral cells were seen in intimate association with nonmyelinated nerve fibers. The proliferated polyhedral cells were identified as ovarian hilus cells.

*Postoperative studies.* In the absence of any overt indication, endocrine studies were not performed prior to operation. Following operation an x-ray film of the skull revealed a normal sella turcica. Urinary 17-ketosteroid excretion was 5.2 mg. per 24 hours. Urinary gonadotropin (FSH) excretion was more than 50 and less than 100 M.U. per 24 hours. These values are normal in this laboratory for a postmenopausal woman.

**Problem: Would you discuss this problem particularly as it relates to hilus cell hyperplasia.**

## Consultation

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Admittedly, it is often difficult to draw a precise distinction between a benign endocrine adenoma and diffuse hyperplasia; nevertheless, we have elected to interpret the hilus cell overgrowth as hyperplasia because it was bilateral and there was no discrete, macroscopic nodule, however small. Hussleín[1] has reported a case in which a discrete hilus cell tumor 4.5 by 3.5 mm. was found in one ovary of a 60-year-old woman who developed adenocarcinoma of the endometrium 25 years after x-ray castration for uterine myomas. Shaw and Dastur[2] have reported 4 cases in which readily detectible clusters of ovarian hilus cells were found in association with endometrial carcinoma; one of their patients, a nullipara 57 years old, had been treated 9 years previously for uterine hemorrhage with 2,400 mg.hr. of radium. In a series of cases of endometrial adenocarcinoma they found ovarian hilus cells to

be more prominent than in the controls. However, it is difficult to determine from their account whether there was a true hyperplasia of hilus cells or whether their observations merely represent the upper limits of a normal observation in the ovaries of a group of postmenopausal women. Significantly, there was no evidence of androgenization in any of the 4 cases they studied in detail, or in Husslein's case, or in the present case.

Simard and Simard[3] describe the case of a 67-year-old woman who developed enlargement and tenderness of the breasts concurrently with disappearance of hot flashes. The clitoris was also enlarged. The ovaries showed not only hyperplasia of hilus cell nests but foci of luteinization of cortical stroma. The endometrium showed cystic glandular hyperplasia. Symptoms regressed following hysterectomy and bilateral salpingo-oophorectomy. They referred to the hilus cells as sympathicotopic cells, and they did not recognize the luteinization as such; however, Scully[4] recognized the luteinization from their photomicrographs, and we concur in his interpretation. In his own case, Scully observed a 7 by 6 mm. nodule of hilus cells in one ovary and a 2 mm. nodule in the other ovary of a 73-year-old woman with adenocarcinoma of the endometrium. There was also patchy, focal luteinization of cortical stroma. The patient exhibited moderate facial hirsutism and the endometrial smear was well cornified. Two years after hysterectomy and bilateral salpingo-oophorectomy, hirsutism and cornification of exfoliated cells were still present.

While hilus cells are occasionally detectible in the ovaries of newborn babies, infants, and children, they apparently increase with age, particularly after the menopause, and are identified more readily in sections of menopausal and postmenopausal women. Prominence of ovarian hilus cells have been observed in such diverse contexts as anencephaly,[5] pregnancy,[6, 7] ovarian dysgenesis with and without masculinization,[8, 9] and choriocarcinoma.[6, 10] Sternberg and associates[6] were able to produce bilateral hyperplasia of hilus cells in the ovaries of adult women following injection of large doses of chorionic gonadotropin. It is interesting to speculate that the hyperplasia of hilus cells in the case reported here may reflect the continuous unopposed stimulation by pituitary gonadotropins at postmenopausal levels over a period of 28 years following pelvic irradiation. However, the gonadotropin level was not very much elevated, and, despite the corroborative support of Husslein's case[1] and Case 3 of Shaw and Dastur,[2] hyperplasia of hilus cells can occur without antecedent pelvic irradiation and subsequent failure of pituitary suppression. The possible relationship of hilus cell proliferation to neurogenic stimuli is suggested by the report by Scully and Cohen[11] of a ganglioneuroma of the adrenal medulla which contained widely distributed hilus cells with crystalloids of Reinke. Such neuroendocrine relationships are reinforced by the common observation that hilus cells are often normally found in close topographic association with nonmyelinated nerve fibers.

Androgenic effects of both tumor and hyperplasia of hilus cells has been reported and well documented by Sternberg.[12] Merrill[13] has tabulated the reported cases of tumor of hilus cell origin. Apparently, in all 10 patients androgenic effects were present to some degree, and most of them showed remission of masculinization following surgical removal of the tumor, but the 17-ketosteroid level was rarely significantly elevated. One of these patients was pregnant, and one of us (W. B. O.) has seen another example of hilus cell tumor of the ovary in pregnancy; it is difficult to reconcile the concept of "masculinization" with the presence of an intrauterine gestation. Clinical androgenization is less constant when there is hyperplasia of the hilus cells as distinguished from true tumor formation. It is possible that some of the reported instances of hyperplasia merely represent the upper limit of a normal cellular constituent; however, it is equally possible that hyperplasia need not be accompanied by parallel hyperfunction. In Scully's case[4] as well as in the case of Simard and Simard[3] there was both clinical and morphologic evi-

dence of hyperestrogenism. It is tempting to assign the increased estrogen production to the patches of luteinized cortical stroma, but chemical proof is lacking in all the cases under discussion, and we must reserve judgment upon which cell elaborates which hormone (if any) until more direct evidence is at hand.

There is some evidence that interstitial cells in the testis can elaborate estrogens. Herrmann and associates[14] cited interstitial cell tumors with gynecomastia as a source of estrogens, and Maddock and Nelson[15] found increase in Leydig cells and elevated urinary estrogens in men treated with chorionic gonadotropin. Scully[16] has reported the case of a postmenopausal woman with polypoid cystic hyperplasia of the endometrium and early adenocarcinoma; one ovary contained a tumor composed of fasciculated spindle cells resembling a thecoma in which there were scattered clusters of hilus cells containing crystalloids and other nests of small epithelial cells consistent with either granulosa cells or Sertoli cells.

The relationship of hilus cell hyperplasia to endometrial adenocarcinoma in this case, or in any other case, is entirely speculative. In the cases of true hilus cell tumor, one patient had normal menses and another was pregnant, but in the postmenopausal patients the endometrium, when reported, was atrophic, appropriate to the patients' age. This is in keeping with the observation by Greene[17] that about 85 per cent of endometrial adenocarcinomas arise in an atrophic, not a hyperplastic, endometrium.

However, the implication inherent in Shaw and Dastur's original communication has been pursued by several observers. Sommers and Meissner[18] found 2 instances of hilus cell hyperplasia in 38 patients with endometrial cancer. Jackson and Dockerty[19] described the ovary in relation to the Stein-Leventhal syndrome; 27 of their patients did not have endometrial neoplasm and were treated by ovariotomy and wedge resection; 16 of their patients had an associated endometrial adenocarcinoma and were treated by hysterectomy and bilateral salpingo-oophorectomy. They observed 5 instances of hilus cell hyperplasia in the 16 patients with endometrial adenocarcinoma, but warn that the two groups are not comparable because tissue from the ovarian hilus was not removed in those patients undergoing wedge resection. Sherman and Woolf[20] found hyperplasia of hilus cells in 81.9 per cent of 133 patients with endometrial adenocarcinoma, in 23 per cent of 52 patients with postmenopausal endometrial hyperplasia, and in only 5.9 per cent of 34 patients with normal atrophic postmenopausal endometrium. They correlated elevated LH levels with these observations, and in 31 of 31 patients (100 per cent) with adenocarcinoma of the endometrium the preoperative urinary LH level was elevated above normal, falling to normal levels in all patients after bilateral oophorectomy. Unfortunately, there was no preoperative indication for endocrine studies in this patient. However, at no time was there any clinical or morphologic evidence of excess estrogenic or androgenic activity. It remains to be demonstrated whether the doctrine of steroid interconversion can be applied to hilus cells, i.e., whether cells which have the capacity to synthesize androgens can under appropriate conditions synthesize estrogens. The total of our knowledge of the hilus cell in this respect is that it is under the control of gonadotropic hormones, probably LH, to some extent, and that when neoplastic it can elaborate androgenic substances, albeit inconstantly. The relation of the hilus cell and its secretions to the pituitary-ovarian relationship and the effect of disturbances of this relationship upon endometrial carcinogenesis merit further investigation.

This case falls into two other oncologic categories which may equally well bear upon carcinogenesis. It is an example of "multiple primary malignant neoplasm."[21] Even though there is no histopathologic documentation of the carcinoma of the bladder for which the patient received x-ray therapy, she did develop an adenocarcinoma of the cecum 16 years later and adenocarcinoma of the endometrium 28 years later. If one cares to speculate upon a constitutional "tumor diath-

esis," this case is as good as any. In fact, it is also a matter for conjecture that this patient may have had as a constitutional factor a greater than average complement of hilus cells as a young girl and that their presence in increased number may have been responsible for the delay of menarche until the age of 18 as well as for their ready postmenopausal overgrowth. Also, she presents an example of "malignant tumor of the uterus developing following pelvic irradiation." The statistical basis for the validity of this mode of carcinogenesis has been debated pro and con,[22-28] but in this case there is the added feature that the adenocarcinoma of the cecum, as well as the adenocarcinoma of the endometrium, might also be attributed to this background. If this hypothesis has any validity, the tumor (or tumors) would be a direct effect of ionizing irradiation and the hilus cell hyperplasia a secondary effect. At this juncture one might speculate that Berenblum's concept of cocarcinogenesis[29] might be applicable. If so, the ionizing radiation would serve as the initiating action and the hypothetical endocrine alteration would serve as the promoting action.

Last, we must remember that in any given patient we do not know the cause of endometrial carcinoma. Indeed, the tumor need not arise from any one given set of pathogenetic circumstances. Adenocarcinoma of the endometrium may develop as the end point of a number of independent pathogenetic pathways. A number of possible mechanisms may, as in the present case, be found in the patient's history or in the pathologic observations. Whether such mechanisms operate independently or in concert is beyond conjecture. Indeed, it is even probable, in the light of present-day ignorance, that the tumor in the uterus arose independently of any of the factors mentioned in the above discussion.

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# Reviews | Abstracts

*Edited by*
LOUIS M. HELLMAN, M.D.

## Selected abstracts

**Acta cytologica**
*Vol. 5, No. 2, March-April, 1961.*

*Graham, Ruth M., Sc.D.: The Small Histocyte: Its Morphology and Significance, p. 77.

**Graham: The Small Histocyte: Its Morphology and Significance,** p. 77.

Patients with similar histologic type tumor and clinical extent of the tumor may have quite different outcomes although treated in an identical manner. This difference has been related to "host resistance." Since the histocyte is an important component of the defense mechanism, this paper is an analysis of the significance of the small histocyte in cancer of the cervix treated by radiation.

Counts of the histocytes were made and their percentage of the total benign cells established.

The paper describes in detail the various morphologic types of small histocytes. It is pointed out that the usually described histocyte with a bean-shaped nucleus was found in only 27 per cent of the total, whereas the oval type nucleus was found in 38 per cent. Although 73 per cent of the cells were round, elongated and bizarre shapes were found in the remaining 27 per cent.

It was found that the majority of the patients with invasive carcinoma of the cervix do not have a large number of histocytes at the tumor bed. However, in the small percentage of patients whose vaginal smears did contain numerous histocytes, the clinical outcome was considerably better than in those in whom the histocytic response was lacking.

*J. Edward Hall*

**Acta obstetrica et gynecologica scandinavica**
*Vol. 40, Suppl. 2, 1961.*

*Ylinen, Olli: Genital Tuberculosis in Women, p. 1.

**Ylinen: Genital Tuberculosis in Women,** p. 1.

The present study was based on 348 proved cases of genital tuberculosis observed in the years 1945 to 1959. The period of observation was 3 years or more in 74 per cent and 6 years or more in 41 per cent. Of 270 married patients, 84 had a barren marriage. More than one third of the patients with pregnancies had an active tuberculous disease within one year after the last delivery. In half of them, genital tuberculosis was proved during that period.

Tuberculous peritonitis at some stage in the course of tuberculous infection was observed in a minimum of 40.5 per cent. A common hematogenous origin from a remote focus, generally situated in the lungs, is to be considered probable for tuberculous peritonitis and for genital tuberculosis in the majority of cases. Pleurisy had existed in 38.1 per cent of the cases with a complete earlier history. In about 3 per cent, uterine hypoplasia may be a result of tuberculous infection in early childhood. This lesion existed in a maximum of 10 to 15 per cent of all cases of genital tuberculosis. Extension to the genital organs occurs in a vast majority of cases at a time corresponding to the age of menarche and to early adult life.

Genital tuberculosis was proved histologically in 77.3 per cent of the cases, histologically and bacteriologically in 18.4 per cent, and bacteriologically in 4.3 per cent. In the whole series, genital tuberculosis was proved through explora-

*These articles have been abstracted.

tory laparotomy in 11.5 per cent and through operative samples of removed parts of the genitals in 43.1 per cent.

A great number of cases of genital tuberculosis, perhaps a majority of them, present few symptoms except the fact of barren marriage. Genital tuberculosis is a local manifestation of a general disease and is in principle a medical disease. Various combinations of conservative drug therapy were used in 180 cases. For such purposes INH and PAS are today the drugs of choice. The length of drug therapy has not been definitely determined. It possibly may be extended from weeks and months to years. The effect of drug therapy on heavy pelvic masses is limited. The latter is an indication for surgical intervention, at least for an exploratory laparotomy. Too early an operative intervention by conservative surgical procedures in acute exacerbations lacking previous treatment with antituberculosis drugs can lead to severe complications. Radical operations were performed in only one eighth of the cases, but are indicated for the most part in patients over the age of 40.

The primary mortality in the whole series was 4.3 per cent, although during the period since 1950 the figure was 1.9 per cent. With few exceptions, fatalities can be avoided. As regards infertility, the prognosis seems nearly hopeless. Not a single case of uterine pregnancy occurred in 290 married patients after proved genital tuberculosis. There were 3 examples of tubal pregnancy after drug therapy.

Indications for surgical intervention may be widened rather than restricted. After the removal of permanently damaged tubes, it is possible to achieve a definite healing of tuberculous endometritis after a moderate treatment with antituberculous drugs. This must often be held as a definite advantage compared with a long-term drug therapy lasting for years. Furthermore, the period of observation can be considerably shortened. Removal of the tubes after a relatively short treatment with antituberculosis drugs would seem more adequate therapy than lengthening the duration of drug administration to cover several years. After bilateral salpingectomy, histological proof of persistent salpingitis was formed in 8 of 38 cases adequately controlled. In all of them ambulatory drug therapy resulted in the healing of the endometrium.

*Robert E. L. Nesbitt, Jr.*

## American Journal of Pathology

*Vol. 38, No. 5, May, 1961.*

*James, J. A., and Ashworth, C. T.: Some Features of Glomerular Filtration and Permeability Revealed by Electron Microscopy After Intraperitoneal Injection of Dextran in Rats, p. 515.

**James and Ashworth: Some Features of Glomerular Filtration and Permeability Revealed by Electron Microscopy After Intraperitoneal Injection of Dextran in Rats,** p. 515.

Young rats were given intraperitoneal injections of dextran twice daily for 14 days; the average molecular weight of the dextran was 75,000 ± 15,000. Light microscopy showed swelling of the glomerular epithelium and an accumulation of fine particles in the foamy, vacuolated cytoplasm. Similar particles were seen in the proximal tubule cells and in histiocytes between the tubules. In electron microscopy, it was found that the glomerular epithelial cells contained aggregates of fine dense particles of about 150 Å. A few of the glomerular endothelial cells contained similar aggregates. The particles were identified, from appearance, size, and PAS staining, as dextran. The glomerular basement membrane appeared to be normal and particles were rarely seen in it. The foot processes of the epithelial cells were intact and narrow.

It is suggested that the accumulation of the large molecules of dextran in the glomerular epithelium may be analogous to the deposition of antibodies and abnormal proteins in this site.

*Leon C. Chesley*

## British Medical Journal

*Vol. 1, Jan. 14, 1961.*

*Benson, P. F., and Joseph, M. C.: Cardiomegaly in a Newborn Due to Placental Chorioangioma, p. 102.

**Benson and Joseph: Cardiomegaly in a Newborn Due to Placental Chorioangioma,** p. 102.

This is a case report of a newborn infant, delivered at 32 weeks' gestation of a 33-year-old para 4-0-2-4, that died in severe respiratory distress at 69 hours of age. Examination of the placenta, which measures 172 by 145 by 50 mm., revealed a chorioangioma, measuring 166 by 100 by 60 mm. attached to the maternal surface of the placenta with a fusiform vessel connecting the chorioangioma to the umbilical vein. The fetus, which weighed 1,360 grams, had a heart weighing 21 grams, or twice the normal size. The

cardiomegaly was due to hypertrophy of all chambers. The histological and anatomical relationships were all normal. The lungs contained a few foci of hemorrhage up to 3 mm. in diameter, especially in the lingula, and areas of intra-alveolar hemorrhage, atelectasis, and scanty hyaline membrane formation. The liver and spleen were both enlarged. The liver canaliculi contained large numbers of bile thrombi. The spleen was normal histologically. The brain had numerous petechial hemorrhages scattered throughout the white matter but no edema was present. Death was attributed to pulmonary syndrome of the newborn and prematurity; however, the case was presented to bring to mind the presence of the chorioangioma as a previously unrecognized cause for cardiomegaly.

*Stuart O. Silverberg*

*Feb. 11, 1961.*

*Wingate, M. B., Meller, H. K., and Ormiston, G.: Acute Bulbar Poliomyelitis in Late Pregnancy, p. 407.

**Wingate, Meller, and Ormiston: Acute Bulbar Poliomyelitis in Late Pregnancy,** p. 407.

A case of acute bulbar poliomyelitis at term resulting in the death of the mother is described.

A para 7-0-1-7, aged 42, was admitted on Aug. 17, 1959, with an expected date of confinement of Aug. 24, 1959. The patient complained of diarrhea and vomiting for 3 days prior to admission, a tight feeling in her throat, and gradually increasing dyspnea. The patient's condition rapidly deteriorated and 4¾ hours after admission she vomited, became cyanosed, and her heart stopped beating. Open cardiac massage was instituted and a classical cesarean section done with delivery of an apparently healthy living female. One and three fourths hours of resuscitation were ineffective in restoring a heart beat. Autopsy revealed lesions typical of acute poliomyelitis and type I virus was grown from the feces, intestinal canal, and spinal cord.

The infant was given 500 mg. of gamma globulin 21 hours after birth. Specimens of stool grew out type I virus until the thirteenth week of life when they became negative. At no time did the infant show any signs of clinical infection. Serial antibody titers in the infant showed very low initial titers which rose in response to gamma globulin with a second much higher rise at 6 months following administration of Salk vaccine.

The literature concerning poliomyelitis in pregnancy and the effect on the fetus and newborn is discussed.

*Stuart O. Silverberg*

*March 4, 1961.*

*Israelsohn, W. J., and Taylor, A. I.: Chromatin-Positive Presumed Klinefelter's Syndrome, p. 633.

**Israelsohn and Taylor: Chromatin-Positive Presumed Klinefelter's Syndrome,** p. 633.

A survey of 1,556 of a total of 1,928 boys with IQ's ranging from 37 to 101, attending schools for educationally subnormal children in the London County Council area, revealed 7 with single-bodied chromatin-positive nuclear patterns and presumed Klinefelter's syndrome (true hermaphroditism excluded by clinical examination). This is an incidence of 0.45 per cent, S.E. 0.17, with 95 per cent confidence limits 0.11 to 0.79 per cent. From these results it is concluded that the incidence of single-bodied chromatin-positive patterns in males does not increase as the intelligence level of the group surveyed falls. Comparisons with the previous surveys taken from the literature are made and the results tabulated and discussed.

It is felt that the information available at this time does not warrant a definite correlation between the incidence of chromatin-positive Klinefelter's syndrome and borderline or subnormal intelligence.

*Stuart O. Silverberg*

*March 11, 1961.*

Huntingford, Peter J.: Intranasal Use of Synthetic Oxytocin in Management of Breast-feeding, p. 709.

Harris, Grahame H.: A Case of Ruptured Uterus and Cardiac Arrest, With Recovery, p. 715.

## Canadian Medical Association Journal

*Vol. 84, No. 16, April 22, 1961.*

Carr, D. H., Barr, M. L., and Plunkett, E. R.: A Probable XXYY Sex Determining Mechanism in a Mentally Defective Male With Klinefelter's Syndrome, p. 873.

*No. 17, April 29, 1961.*

Robinson, S. C.: Observations on Vaginal Trichomoniasis. I. In Pregnancy, p. 948.

## Journal of the American Geriatrics Society

*Vol. 9, March, 1961.*

*Nielsen, R. L., Moore, D., and Paulsen, C. A.: Urinary Estrogen and Gonadotropin Excretion in Elderly Women and the Plasma Corticoid Response to Corticotropin, p. 178.

**Nielsen, Moore, and Paulsen: Urinary Estrogen and Gonadotropin Excretion in Elderly Women and the Plasma Corticoid Response to Corticotropin,** p. 178.

Studies were performed on 18 women who were between the ages of 76 and 91 years (median: 82 years). All were at least one month convalescent from various traumatic episodes and were otherwise well. None had undergone pelvic operation or irradiation and none had any complicating illnesses except that several had well-compensated heart disease that was being treated with digitalis. All were mentally competent. The concentration of plasma corticoids was found to range from 9.5 to 40.8 μg per 100 ml. (arithmetical mean 18.6 μg). Following the intravenous administration of 25 units of ACTH the average rise in concentration was 29.4 μg per 100 ml. (range 18.3 to 38.1 μg). In none of the patients was the rise less than normal but in 4 there was some degree of hyperresponsiveness although there was nothing to suggest Cushing's syndrome in the 4 women.

The urinary estrogen excretion was low in that the mean value was 0.26 ± 0.05 (S.E.) μg equivalents estradiol per 24 hours. Six of the women had no estrogenic substance that was detected by the method used. Osteoporosis as shown by vertebral deformity was present in 6 of the 18 women. The output of estrogen was not determined in one of these women but in each of the others the output of estrogens was so low as to be not detected.

The excretion of urinary gonadotropins varies from 0.45 to 7.3 mg. equivalents HMG-J-5 per 24 hours. In all but 3 women the excretion of gonadotropins was increased.

There was no correlation between the age of the patients and the amount of either estrogen or gonadotropins that were excreted. However, the amount of estrogens that were being excreted was found to be lower than the value of 0.35 ± 0.04 μg equivalents that had been previously reported for a group of younger postmenopausal women with a median age of 58 years (J. Am. Geriatrics Soc. 6: 903, 1958). This together with the observation that no detectable estrogen is excreted by surgically castrated women suggests that despite the lack of correlation there may be a decline in estrogen production with advancing years.

*David M. Kydd*

## Journal of Clinical Endocrinology and Metabolism

*Vol. 21, No. 2, February, 1961.*

*Savard, K., Gut, M., Dorfman, R. I., Gabrilove, J. L., and Soffer, L. J.: Formation of Androgens by Human Arrhenoblastoma Tissue in Vitro, p. 165.

**Savard et al: Formation of Androgens by Human Arrhenoblastoma Tissue in Vitro,** p. 165.

Arrhenoblastoma tissue slices were incubated in vitro with progesterone 4-C14. The radioactive products isolated were 20-hydroxy-4-pregnen-3-one, 20-hydroxy-4-pregnen-3-one, 17-hydroxyprogesterone, 4-androstene-3,17-dione, and testosterone. No estrogen was formed and there was no evidence of the presence of the enzyme 11-hydroxylase. Considerable significance is attached to the demonstration of the biosynthesis in this tumor tissue of the very potent androgen testosterone.

The authors lay great stress to the importance of finding testosterone in arrhenoblastoma tissue. It is known that androstenedione is found in normal ovarian tissue and that this hormone is also elaborated in androgen-producing tumors. However, it is well known that many arrhenoblastomas do not have a marked virilizing effect and other tumors of the same type do have considerable virilization, but with a relatively low urinary 17-ketosteroid. It is suggested that virilization is to a large degree related to the amount of testosterone produced by the tumor rather than the other androgens which may be found. The ability of small amounts of testosterone to produce virilization is well known and therefore it is understandable that the elevation of 17-ketosteroids does not need to be high in order to have virilizing effects.

*J. Edward Hall*

## Journal of Obstetrics and Gynaecology (New Delhi)

*Vol. 21, April, 1960.*

*Salacz, P. E., and Salacz, T. S.: Vaginal Delivery Following Cesarean Section, p. 73.

**Salacz and Salacz: Vaginal Delivery Following Cesarean Section,** p. 73.

The authors have reviewed the literature regarding rupture of previous cesarean section scars and have tried to evaluate two questions which arise: (1) to what extent is such a woman endangered by the scar in the uterus? and (2) what prospect has she for a vaginal delivery at term? In 241 cesarean sections, of which 39 (16.2 per cent) were repeat cesarean sections, there was one maternal death in a primary section and no deaths in the repeat sections. The uncorrected perinatal fetal mortality for all cesarean sections was 7 per cent (17 infants) and none for repeat sections. They had no disruptions of scars in either the repeat cesarean section group or in those delivered vaginally following previous cesarean section.

Thirty-three women were delivered thirty-six times per vaginam following cesarean section. The only fetus lost was a premature infant of 1,100 grams. The uterine cavity was explored in each case and found to be intact. The puerperium was normal in 33 cases and febrile in 3 cases. The conclusions arrived at by the authors is that with due foresight and careful observation of the patient and an adequate knowledge of the conditions and techniques of the primary cesarean section, with a well equipped hospital, a patient may be allowed to be delivered vaginally as long as the original indication for cesarean section is no longer present. They advise against the use of oxytocics and recommend routine exploration of the uterus after delivery. *Stuart O. Silverberg*

## Journal of Obstetrics and Gynecology of India

*Vol. 11, September, 1960.*

*Malkani, Parvati K., and Mirchandani, Janki J.: Menstruation During Lactation, p. 11.

**Malkani and Mirchandani: Menstruation During Lactation,** p. 11.

The authors have studied 390 lactating women to determine the duration of amenorrhea associated with lactation and its effectiveness as a barrier to further pregnancy. Of the 390 lactating women, 108 women were still amenorrheic at the completion of the study. Of the 282 remaining, 81 per cent had commenced menstruation while still lactating. The mean duration of lactation was 13.5 months. The mean duration of amenorrhea was 5.25 months. The duration of amenorrhea increased as the duration of lactation increased: those who weaned early had early menstruation. The duration of amenorrhea was longer if supplementary milk was not given to the infant: a greater proportion of the partly lactating mothers started menstruating earlier than the fully lactating mothers. The earlier the artificial milk supplement was started, the earlier menstruation commenced. The duration of amenorrhea increased with age, and also with parity and decreased as the per capita income increased. This latter was correlated by the authors with the better nutritional status of the higher income group.

In the absence of lactation, the mean duration of amenorrhea was related to the duration of gestation as follows: Full term, 58 days; premature delivery, 42 days; 13 to 27 weeks' gestation, 38 days; and 6 to 12 weeks' gestation, 31 days. *Stuart O. Silverberg*

## Lancet

*Vol. 1, Feb. 4, 1961.*

*Wielenga, G., van Tongeren, H. A. E., Ferguson, A. H., and van Rijssel, Th. G.: Prenatal Infection With Vaccinia Virus, p. 258.

*Shearman, R. P., Cox, R. I., and Gannon, A.: Urinary Pregnanetriolone in the Diagnosis of the Stein-Leventhal Snydrome, p. 260.

**Wielenga et al.: Prenatal Infection With Vaccinia Virus,** p. 258.

At the time when a woman aged 19, who had not been vaccinated and who bore no scars of vaccination, was 18 weeks pregnant, her 11-month-old son was vaccinated. Within a week the son developed a reaction with vesicles which ruptured in 2 weeks. The mother developed a slight sore throat with fever 15 days after her son was vaccinated. When the pregnancy reached about the twenty-seventh week the woman gave birth to a premature infant which died within 10 minutes. The baby had extensive skin lesions that immediately suggested vaccinia. Autopsy showed lesions on several organs and the placenta had many necrotic villi. Material from the skin lesions and placenta was cultured in the chorioallantoic membranes of 12-day-old incubated hens' eggs. Elementary bodies typical of the pox viruses were found after 72 hours. In serum obtained from the mother 8 days after delivery vaccinia-virus neutralizing antibodies at a high titer were found.

The possibility that this previously unvacci-

nated woman developed an airborne infection from her older son and that the virus infected the placenta causing the fetus to develop fatal viremia is suggested. The assumption that vaccination causes no damage to the fetus after the thirteenth week of pregnancy appears to be untenable.

*David M. Kydd*

**Shearman, Cox, and Gannon: Urinary Pregnanetriolone in the Diagnosis of the Stein-Leventhal Syndrome,** p. 260.

In 16 consecutive patients with Stein-Leventhal syndrome the daily excretion of 17-ketosteroids, 17-ketogenic steroids, pregnanediol, and pregnanetriol was found to be in amounts that normal women excrete. However, in each of these instances pregnanetriolone was present in quantities of 8 to 260 $\mu$g per 24 hours. This substance was not detected in the urine of normal women. It is found in patients with adrenal hyperplasia but in this condition there are also abnormalities in the excretion of 17-ketosteroids or 17-ketogenic steroids.

This preliminary report suggests that urinary pregnanetriolone estimations may be a useful aid in the diagnosis of the Stein-Leventhal syndrome and in deciding which women will benefit from a wedge-resection operation.

*David M. Kydd*

# Correspondence

## Superfetation

*To the Editors:*

The paper by Weinberg (Am. J. Obst. & Gynec. 82: 226, 1961) describing a case that might possibly be one of superfetation prompts me to direct your attention to a case report with somewhat similar findings of interest entitled, "Coexistent Pregnancy of Unequal Twins" (A. M. A. J. Dis. Child. 95: 649, 1958).

The case report tells of the birth of "a set of live-born dizygotic twins weighing 2 lb., 8 oz. and 6 lb. respectively, with a marked dissimilarity in size and maturity. . . ." The pregnancy had been complicated during the second month by a nephrectomy for renal calculus. The placenta was divided into two parts and there were two sets of membranes. Roentgenograms showed the larger twin to have well-visualized centers of ossification in the epiphysis at the distal end of the femur and at the proximal end of the tibia. The smaller twin had beginning ossification of the distal femoral epiphysis and absence of ossification at the proximal tibial epiphysis. The larger twin's bone age was interpreted as normal for the average full-term male infant, while the smaller twin's bone age was considered "comparable to that usually seen in male infants 4 to 6 weeks before term."

The authors felt that the disparity between the twins could be attributed to a difference in nutrition of the two fetuses resulting from the placental variations rather than to the phenomenon of superfetation.

*George X. Trimble, M.D.*
*Director of Medical Education*

*Memorial Hospital of Long Beach*
*Long Beach, California*
*July 10, 1961*

## Reply by Dr. Weinberg

*To the Editors:*

In answer to Dr. Trimble's letter of comment on the article on superfetation, I was very interested in his reference in the *American Journal of Diseases in Children.*

I would also like to call attention to the fact that Dr. John Kelly of the University of California wrote me and called attention to an article by Cope and Murdock in the *Journal of Obstetrics and Gynaecology of the British Empire,* Vol. 65, p. 56, 1958.

It has become evident that the only proof of whether or not bone age disparity is of any value will be with a controlled study with a large series of twins. It would also be of help if we can get a large series of proved monozygotic twins by a section of the fetal membrane showing that they are mono- or dichorionic and seeing if any group of monochorionic twins has bone age disparity.

*Paul C. Weinberg, M.D.*

*2525 Eutaw Place*
*Baltimore 17, Maryland*
*July 26, 1961*

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## Isoxsuprine and duration of labor in primigravidas

*To the Editors:*

A certain percentage of our patients in premature labor treated with intravenous isoxsuprine (Vasodilan, supplied by Mead Johnson Laboratories) had a marked relaxation of the lower uterine segment with a rapid early delivery. This led one of us (M. J. W.) to believe that intravenous isoxsuprine might favorably alter the course of active labor in the primigravida.

Fifty consecutive primigravidas who met the following criteria were studied: in active labor with the cervix dilated not over 4 cm., a single fetus in vertex presentation, and normal clinical pelvimetry findings. No form of anesthesia had previously been used. A vaginal examination was done and an amniotomy followed if the membranes were not ruptured. Twenty milligrams of isoxsuprine was then given intravenously in 500 ml. of 5 per cent glucose in water at the

Table I

| *No. of cases* | *Time from 2 to 4 cm. dilatation to delivery* | *Contractions stopped or became weaker* | *Babies weighing over 3,600 grams* |
|---|---|---|---|
| 37 | 43 to 299 minutes | 0 | 8 |
| 2 | 300 to 359 minutes | 1 | 1 |
| 7 | 360 to 479 minutes | 4 | 2 |
| 4 | 480 and over | 1 | 3 |

rate of 50 to 60 drops per minute. The findings regarding cervical effacement, thickness, station, and uterine contractions were recorded. Vaginal examination was repeated at 20, 40, and 60 minutes and then as often as deemed necessary with appropriate recording of data by two of us. An analgesic, usually meperidine, was given as needed. A subarachnoid block was used for most deliveries; in nearly all the rest a pudendal block was administered.

Within the first 20 to 40 minutes after isoxsuprine was started, cervical softening was noted in 90 per cent of the patients. There were 9 patients who had persistent posterior presentations. Their average time from 2 to 4 cm. cervical dilatation to delivery was 2 hours, 53 minutes. The average time for the 50 patients was 3 hours, 49 minutes. In the group who were delivered in under 5 hours and had strong regular contractions, 3 were delivered in 1 hour or less, 14 in 1 to 2 hours, 7 in 2 to 3 hours, 8 in 3 to 4 hours, and 5 in 4 to 5 hours. The average time for these was 2 hours, 34 minutes. Thirteen patients were delivered in from 5 to 11 hours. Of these, 5 had babies weighing more than 3,746 grams, 1 had a persistent posterior presentation, and in 6 the contractions either ceased or became weak and irregular for 1 to 6 hours (Table I). No evidence of hypotension, tachycardia, or other toxic manifestations was noted in the mother or the newborn infant. Pain appeared to be lessened and only 4 patients required more than one injection of an analgesic. All babies by Apgar score were rated 8 or better.

In premature labor both Bishop[2] and we[1] have noted that isoxsuprine when given in a more concentrated solution appears to stop uterine activity within a few minutes. There is evidence that it can also do this in normal labor.[1] It is suggested that the dosage employed here apparently has a greater effect upon relaxation of the lower uterine segment than it does on myometrial activity. We are now attempting to verify this in vitro. It is assumed that where uterine contractions ceased for 3 to 6 hours after isoxsuprine is given we have exceeded the threshold for those patients. In order to obviate the influence of amniotomy as well as other extraneous factors, we are now doing a random blind study. We are also investigating the possible uses of isoxsuprine for induction of labor in both primigravidas and multigravidas with a medical indication as well as in patients who have had a previous cesarean section for causes other than dystocia. There is some reason to believe that it may be effective in elective induction in multiparas[3] although we have had no experience with this. It is assumed that the excellent condition of the newborn infant was due to little analgesia and a short labor.

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3. Banks, A. L.: Personal communication, 1961.

*M. James Whitelaw, M.D.*
*H. Bertelsen, M.D.*
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*Department of Obstetrics & Gynecology*
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Item

**South Western Obstetrical and Gynaecological Society**

The autumn meeting of the South Western Obstetrical and Gynaecological Society will be held at the Portsmouth Guildhall, Nov. 7, 8, 9, and 10, 1961. There will be 5 half-day sessions. Subjects will include: Unstable Presentation; Obstetric Anesthesia; The Place of the General Practitioner in Obstetrics; Asphyxia Neonatorum; Management of the Third Stage. Registrations and further information are obtainable from the Secretary of the Society, St. Mary's Hospital, Portsmouth, England.